

Investigation of impacts of underwater explosions on larval and early juvenile fishes

Part 1:

The effects of underwater explosions on larval fish
with implications for the Wilmington Harbor Project

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Part 2:

Trauma to late-stage larval and early-juvenile pinfish, *Lagodon rhomboides*, and spot,
Leiostomus xanthurus, inflicted by sub-marine detonations.

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Part 3:

Instrumentation report

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Abstract

The Cape Fear River ship channel is being deepened by an average of 1.2 m in order to accommodate deep draft ships. Because of the presence of rock in portions of the harbor, blasting is required to achieve project depth. The blasting plan calls for a total of 725 blasts over a 5-7 year period to remove 520,693 m³ of rock covering 303,521 m² of river bottom .

We conducted a controlled field experiment to (1) to determine sensitivities of larval and recently metamorphosed spot, *Leiostomus xanthurus*, and pinfish, *Lagodon rhomboides*, to shockwave exposure; (2) to determine the type and frequency of fish injury; (3) to develop empirical models relating fish injury to shockwave exposure; (4) to estimate the number of larval and early juvenile fish injured by blasting in the Wilmington Harbor; and (5) to provide recommendations for appropriate field monitoring locations.

Fish were exposed to small underwater explosions at three distances from the blast. Shockwaves were monitored using pressure transducers and digitally recorded. Injuries were assessed using both gross observation and histology. Spot suffered both greater injury and mortality. Injuries were observed to the kidney, swimbladder, liver, and pancreas. For both species, the most common shockwave-induced injury was hematuria (i.e. damage to the tubules of the kidney). Others injuries included hemorrhage within the coelom, swimbladder hemorrhage, liver hemorrhage, coagulative liver necrosis and ruptured pancreas.

The use of explosives to aid in the removal of rock in the Cape Fear River will undoubtedly result in injury and mortality of some organisms including larval fishes. Results from this study suggest that nearly 8.2×10^8 larvae could be injured or killed over the duration of the project. While that is an appreciable number of larvae, it represents only 2.3-3.2 % of the larvae in the system during all the August-January blasting windows combined over the life of the project. Such a low level of impact is unlikely to affect the local population. Based on our results, it is recommended that the near-surface impulse should not exceed 7 Pa s at a distance of 210 m.

Introduction

Concern about impacts of the detonation of underwater explosives (i.e. blasting) to living resources is focused on mortality of fish, turtles and marine mammals. This focus results from the public interest in these animals and their relatively high sensitivity to rapid pressure changes generated by underwater explosions. While limited numbers of other species will be injured or killed by blasting, animals with gas-filled internal organs (i.e., lungs or swimbladders) are vulnerable at a greater distance.

The level of injury and mortality can be predicted from theoretical or empirical models. Unfortunately predictions of injury and mortality are not very precise for a variety of reasons including: (1) uncertainty about distributions (i.e., how many individuals will be exposed, at what depth, and at what distance from the blast); and (2) species-specific and size-specific differences in sensitivity to shockwaves.

The Cape Fear River ship channel is being deepened by an average of 1.2 m in order to accommodate deep-draft ships. Because of the presence of rock in portions of the harbor, blasting is required to achieve project depth. The blasting plan calls for 1 to 6 blasts per day for a total of 725 blasts to remove 520,693 m³ of rock covering 303,521 m² of river bottom (USACE, 2000a). The blasting schedule extends from August through January. Blasting will utilize buried and stemmed charges with time-delayed detonations to minimize shockwaves. Natural resource managers recommended that blast impacts be monitored (USACE, 2000b).

Assuming that risks to protected species (i.e., sturgeon, sea turtles, marine mammals) are minimized by the blasting schedule, fish mortality is the primary concern of the project. Even with extensive methods to reduce impacts, blasting will still result in some mortality of adult,

juvenile, and larval fish. Existing models for prediction of lethal impacts on large juvenile and adult fishes are fairly robust and total mortality can be predicted with reasonable accuracy, provided fish abundance and distribution are known (Yelverton et al., 1975; Goertner, 1978; Wiley et al., 1981; O’Keeffe, 1984; Munday et al., 1986). Unfortunately, the abundance and distribution of late-stage juvenile and adult fishes are both highly variable in time and space. If we assume, however, that estimates of the lethal area surrounding the blast are accurate, and that the blast does not affect areas of special concentration (e.g., spawning aggregation), the conclusion that blasting has no significant adverse impact to late-juvenile and adult fishes seems reasonable. Estimation of actual blast-induced mortality on these populations would be expensive and of little management value. Limited sampling immediately after a few blasts is sufficient to document the relative size and species composition of mortalities. The impacts to adult and juvenile fish were adequately addressed based on existing literature, and test blasting using caged fish in Wilmington Harbor during the winter of 1998/1999 (USACE, 2000a), however, larval fish were not used during test blasting, and scant information is available on the impacts of blasting on larvae.

Wright (1982) suggested that larval fish might be less sensitive to the effects of shockwaves than juvenile and adult fish, but the limited evidence suggest otherwise. A number of studies have found increased sensitivity to blasting with decreasing fish size (e.g., Yelverton et al., 1975; Goertner, 1978; Wiley et al., 1981; O’Keeffe, 1984; Munday et al., 1986). The general relationship between fish size and sensitivity is largely based on observations of more advanced juvenile stages. Extrapolation beyond the conditions tested is always questionable, and in this case, because larvae are generally more sensitive to stress than more advanced

developmental stages, prediction of impacts in the absence of empirical data is completely inappropriate.

Potential deleterious effects to early-life-history stages of fishes are in need of study because large numbers of larvae and early post-larval juvenile fishes can be exposed and Fitch and Young (1948), Kostyuchenko (1972) and Nix and Chapman (1985) have reported larval mortality due to underwater explosions. Neither the sensitivity of larvae nor inter-specific variation in larval sensitivity has been sufficiently examined. Bishai (1961) found that young brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) were killed when exposed to peak pressures exceeding 200 kPa, while larval herring (*Clupea harengus*), lacking a swimbladder were not. Although these data are limited, it is very important in the context of the Wilmington Harbor project as the blasting plan calls for peak pressures to average 483 kPa and not exceed 827 kPa at a distance of 43 m from the detonation site (USACE, 2000a). Clearly, there exists the potential for significant impacts to larval fishes with developed swimbladders. Because blasting will occur within a rather narrow migratory pathway (i.e., the river channel) for these fishes (Lawler et al., 1988), a substantial fraction of the local population could be at risk.

The task of determining larval fish mortality due to blasting through field studies is complicated by several factors: (1) the harsh environment (wind, waves and currents) which make field studies difficult; (2) methods for precise and unbiased sampling of larvae are not routine and standard, thus field estimates of abundance, distribution or condition must also include some evaluation of sampling methodology; (3) most larvae are very sensitive to disturbance so that handling effects may be confounded with blasting impacts; and (4) sub-lethal effects of blasting may impair the larvae's ability to avoid predation. Because of these

complicating factors controlled experiments and a modeling approach to predict larval sensitivity is more useful.

The objectives of this study are (1) to determine species-specific sensitivities of larvae to shockwave exposure under experimental conditions; (2) to determine the type and frequency of injury; (3) to develop empirical models relating fish injury to shockwave exposure; (4) to estimate the number of larval and early juvenile fish injured by production blasting in the Wilmington Harbor; and (5) to provide recommendations for appropriate field monitoring locations during production blasting.

Methods

Experimental design - overview:

Late-stage larval and recently metamorphosed fishes were exposed to underwater shockwaves resulting from small detonations under controlled conditions to determine sensitivity to injury. Fish of two species were placed at 3.6 m, 7.5 m and 17 m from the blast: three replicates plus a control were performed at each distance, for each species (Table 1). After exposure, fish were observed for signs of injury and mortality at three time intervals over a 24 h period. Injury assessment was conducted using standard histological techniques.

Study Area:

The field experiment was conducted March 20-21, 2001 in Pivers Island channel adjacent to the NOAA Center for Coastal Fisheries and Habitat Research in Beaufort, North Carolina (Fig. 1). The channel is about 30 m wide and 6 m deep.

Underwater detonations:

A series of small underwater explosions were conducted using 12-grain Primadet PDT 1403 detonators. Shockwaves were monitored using PCB 138A01 pressure transducers and digitally recorded with a Gage 512-1M at 5 million samples per second and a resolution of 12 bits. All detonations during the testing of impacts on young fish were conducted at a depth of 2 m in the center of the channel from a research platform suspended from a bridge that crosses the channel. Details of the instrument configuration and shockwave characteristics are provided in Lynch and Revy, (2002).

To determine the approximate 6.9 Pa s (1 psi-msec) impulse boundary, a predicted threshold for lethal impact to young fish (Yelverton et al., 1975), preliminary pressure readings were recorded from an anchored boat at various distances from the blast. During these preliminary tests, pressure sensors were deployed simultaneously at the same depth and distance for each blast. To determine the effect of the bag on the underwater shockwave, one sensor was located in a water filled plastic bag that would contain larvae in future blasts, while the second sensor was located outside of the bag. Although the bags reduced the shockwave an average of 22.4%, the level of exposure that the test fish would experience would be sufficient to evaluate shockwave-induced injury (see Lynch and Revy, 2002).

Three characteristics of underwater shockwaves were examined, each of which has previously been used to predict the impact of blasting to aquatic animals (MacLennan and Simmonds, 1992): (1) peak pressure (P_{\max}) - the maximum pressure relative to hydrostatic pressure generated by an underwater shock wave with units of pascals (Pa) ; (2) specific impulse (I) - the time-integral of the pressure of a shock wave with units of pascal seconds (Pa s) ; and (3)

energy flux density (EFD) - the rate of energy transport across a unit area with units of joules per square meter (J m^{-2}).

$$I(t) = \int_0^t P(t) dt$$

$$EFD = \frac{1}{\rho c} \int_0^t P^2 dt$$

where: P is the shock wave pressure
 ρ is the density of the water
 c is the speed of sound in water

Hydrographic measurements:

Hydrographic profiles of the water column in the channel were taken prior to each deployment of fish and subsequent detonation using a Sea-bird Electronics[®] CTD-19. The instrument recorded hydrostatic pressure, temperature and conductivity, from which salinity, seawater density and sound velocity were derived. Post processing to align each sensor's response times followed the manufacturers recommendation. Data were binned into 1 m averages and temporal contour plots were generated with SURFER 7[®] using the kriging method without smoothing. Sampling was conducted over a tidal cycle, which affected the temperature and salinity structure of the water column (Fig. 2), and by extension, seawater density and speed of sound propagation (Fig. 3), both of which effect characteristics of underwater shockwaves.

Handling and deployment of fish:

A week prior to the field experiment, several thousand larval and early-juvenile fishes of several species were collected from the channel using a 2x2 m plankton net equipped with an 8 m long net with 0.947 mm mesh. A live-box was attached to the cod-end so that fish could be

removed alive with minimal stress (Hettler, 1979). These fish were transferred to a 2000 l holding tank that received filtered ambient seawater. Fish were maintained on a natural light cycle and fed COREY HI-PRO Starter Feed[®] *ad libitum*. Spot, *Leiostomus xanthurus*, Atlantic menhaden, *Brevoortia tyrannus*, and pinfish, *Lagodon rhomboides*, were the dominant taxa represented. Unfortunately, most of the menhaden did not survive, most likely due to the effects of handling.

Twenty-four hours before the field experiment, several hundred spot and pinfish were removed from the large holding tank to the controlled environment of the larval fish rearing facility and maintained in two 50 l tanks with flow-through filtered ambient seawater. Fish were gently transferred from these holding tanks into the 24 test chambers. Each test chamber consisted of a 24x36 inch round-bottom polyethylene bag of 2 mil thickness. Bags were filled with 5 l of filtered ambient seawater and individually placed in 11 - 19 l plastic buckets. Twelve bags contained between 20-30 spot and 12 bags housed 20-30 pinfish. All bags were supplied with aeration and maintained in a flow-through water bath of ambient seawater under a 12h:12h light:dark regime. All dead and obviously distressed fish were removed from the test chambers prior to their deployment which resulted in the bags containing 15-30 fish.

Fish were fed *ad libitum* the day before the field experiment. To ensure optimal water quality in each bag for the remainder of the 24 h observation period, 3 liters of seawater were decanted from each bag and replaced with 3 liters of filtered ambient seawater immediately following the 4 h observation period. A food ration was added to each bag after the water change. Rations were again provided in the early morning and late afternoon the following day.

A Hydrolab[®] H20 was used to monitor seawater temperature and dissolved oxygen (DO) in the bags prior to deployment, immediately after return the lab and again at each subsequent observation period.

The selection of the locations were based on preliminary detonations and shockwave pressure measurements taken the day before (see Lynch and Revy, 2002). The shockwave field was thought to encompass a range of potential effects on the fish ranging from mortality to 100 % survival. Distance of deployment was chosen randomly. Once a distance was selected, the four treatments were deployed in random order: pinfish and spot with detonation (n=3) and pinfish and spot with no detonation (n=1). The depth of all the bags and the detonation was 2 m.

A 6 m boat was positioned on sight using a laser rangefinder and secured in position with a 3-point anchor array so that the bow was directly in line with the shockwave source (Fig. 4). Two waterproof coax cables connecting the pressure sensors and the signal recording equipment extended from the bridge platform to the boat. A second small boat ferried 2 bags (1 of each species), still in the buckets, to the anchored vessel. Each bag was then removed from the bucket and the pressure sensor was suspended vertically in the center of the bag. The bag was then secured with a wire-tie and placed inside a 0.5 m circular net frame equipped with a loosely-fitted purse constructed of 3-inch monofilament netting. The purse was closed and the frame lowered to a depth of 2 m with the open face of the net frame perpendicular to the explosive charge (Fig. 4). One test array was deployed off the starboard bow and one off the port bow. The net frames were held in position with c-clamps and fore and aft stays. Total time elapsed for the deployment of each bag was usually less than five minutes. Once the bags were in place and all clear was sounded, the underwater charge was detonated. The bags were immediately retrieved

and initial observations were made on the number of dead or injured fish in each bag. The bags were returned to the buckets and to the standby boat and then returned to the laboratory. The next set of bags were then delivered to the field.

After the completion of the field experiment, an additional 50 spot and 50 pinfish were sampled from the 2000 l holding tank and killed with MS-222. The standard length (SL) was measured before fixation in 10% formalin and again at two weeks after preservation. Linear regressions were used to correct SL of the experimental fish to SL in life:

$$\text{spot } SL_{\text{life}} = 0.3867 + 0.9572 SL_{\text{fixed}}$$

$$\text{pinfish } SL_{\text{life}} = 0.5325 + 0.9408 SL_{\text{fixed}}$$

Injury Assessment:

Once in the lab, bags were examined again for dead or injured fish. Dead fish were removed, examined for signs of gross injury with a stereo microscope, measured, and preserved in 10% histological-grade neutral-buffered-formalin. Fish that showed signs of injury (i.e. disoriented or aberrant swimming) were gently removed from the bags and examined with a dissecting scope at 10x - 50x magnification. If no obvious injury was detected during these gross anatomical examinations the fish were returned to the bag. All test bags were examined in the lab again 4 h and 24 h after field exposure. Fish that were dead or injured were treated as above. At the end of the 24 h observation period all remaining fish were killed with MS-222 and fixed for histology.

Histological injury assessment was conducted on a sub-sample from all bags. Standard techniques were used including embedding in paraffin, sagittal sectioning (5 micrometer thick

sections) and staining with standard stains. The selection of specimens for histology was as follows: (1) all fish that died during the 24 h observation period, (2) all fish that appeared injured during the final gross anatomical exam at 24 h post-field exposure, and (3) a random selection of 6 additional fish from each bag.

As with most previous investigations of blast-induced impacts on fishes, we adopted the scaled damage (i.e. injury) criteria of Hubbs et al. (1960) (Table 2). The following assumptions were made concerning interpretation of lethal and sub-lethal injury: (1) rupture of visceral organs is lethal, (2) severe hemorrhage and/or necrosis in visceral organs is lethal, (3) minor hemorrhage in the coelom is sub-lethal, and (4) minor kidney damage is sub-lethal. Prior observations support these assumptions. While fish with minor hemorrhage to the kidney and swimbladder appear to swim normally (Yelverton et al., 1975), even slight damage to the kidney is likely to reduce a fishes osmoregulatory efficiency and increase energy expenditure (Gaspin et al., 1976). Fish with level 2 or 3 injuries may survive under captive maintenance (Yelverton et al., 1975), but fish sustaining an injury to the kidney, swimbladder, liver or other major organs equal to Level 2 or greater are unlikely to survive in nature (Cronin, 1948; Gaspin et al., 1976; Wiley and Wilson, 1975; Wiley et al., 1981).

Data Analysis:

Linear and non-linear regression was used to relate proportion of a species injured, both lethally and sub-lethally to P, I and EFD. These empirical models were used to estimate blasting impact to fish assessed as a lethal dose (LD), sub-lethal dose (SLD) and total injury (i.e., lethal + sub-lethal) dose (TID) at the 1%, 10% and 50% levels (i.e. LD₁, LD₁₀, LD₅₀, SLD₁, SLD₁₀, SLD₅₀,

TID₁, TID₁₀ and TID₅₀).

The experimentally-derived relation between shockwave parameters and larval injury were then used to predict the number of larvae that will be injured by the Wilmington Harbor blasting project. Production shockwave monitoring data from the Cape Fear River were obtained from the Appendix B in Rickman (2000). Data were averaged for each nominal depth bin (0.9 m, 4.6 m, and 9.1 m) and at each of 5 distances (10.7 m, 21.3 m, 42.7 m, 85.3 m, and 170.7 m) from the detonations. These means were used to characterize the shockwave field (i.e. P, I, EFD) along the radius of a circle around a production blast. Using the above estimated LD, SLD and TID values, a conservative estimate of the lethal and sub-lethal range (r) was obtained as the most distant point down range, at any depth, where the value was exceeded. The volume (v) of water associated with these LD, SLD and TID values was estimated using

$$v = \pi r^2 h \text{ when } r < 125 \text{ m}$$

$$v = 2rwh \text{ when } r > 125 \text{ m}$$

where: r = maximum horizontal range associated specified LD, SLD and TID levels

w = nominal width of river channel = 125 m

h = nominal river depth = 13.7 m

Information on the concentrations of larval and early-juvenile fishes that inhabit the river in the vicinity of the rock blasting, during the blasting season (August-January) were obtained from CP&L Brunswick Steam Electric Plant Environmental Monitoring Reports. Larval fish concentration data, in concert with the above estimates of the volumes of water impacted by blasting, were used to estimate the average and worst-case impact per blast on resident young fish. These estimates rely on the assumptions that fish are equally distributed per unit volume

throughout the water column in the affected area and the impact at maximum horizontal range is equal throughout the water column for a prescribed LD, SLD and TID level. Neither of these assumptions are likely to be valid, but such a conservative treatment provides a useful first-approximation appropriate when taking a precautionary approach to resource protection.

Results

Field experiment injury assessment:

A total of 232 spot (175 exposed:57 control) with mean SL ranging from 18.0 to 20.1 mm and 251 pinfish (190 exposed and 61 control) with mean SL ranging from 15.9 to 17.2 mm were used in the field experiment (Table 1). Seawater temperature and DO remained stable in the bags throughout the experiment. Handling time, the time from removal of a bag from the lab until its return, averaged 57.8 min. Submergence time, the time a bag was at 2 m, averaged 12.8 min. On two occasions these times were prolonged due to detonation failure.

P_{\max} , I and EFD were highest closest to the blast (Table 3). There was, however, considerable variability in the parameters between replicates. Mean values (n=6 at each location), however, exhibited an expected exponential decay with distance (Figs. 5-7).

Spot and pinfish differed in their sensitivity to shockwaves. Fish mortality and gross injury over the 24 h observation period are provided in Table 3. Of note is the limited mortality or injury observed in pinfish. Five pinfish exposed to blast shockwaves died during the 24 h observation period. Two of these fish were from the closest station to the blast (i.e. 3.6 m): one found at 4 h had been eviscerated and the other found at 24 h was observed to have hemorrhaging dorsal to the swimbladder and ventral to the kidney during gross examination. The other three

pinfish were dead at 24 h and all were from the 7.5 m station. Three of the five dead pinfish had a poor reaction to the histological stains due to autolysis. There was no mortality among the control pinfish.

Spot suffered both greater mortality and injury. Twenty exposed spot died: one found immediately following exposure (i.e. 0 h), two at 4 h and 17 at 24 h. Of these, two showed signs of hemorrhage dorsal to the swimbladder in the region of the kidney upon gross examination and both were exposed at the station closest to the blast. Six other spot were eviscerated by cage-mates. The remaining 14 showed no signs of trauma during gross examination. Unfortunately, most of these fish (17 of 20) had undergone some autolysis by the time of fixation which contributed to a poor reaction to the histological stains. Although kidney damage could be detected in two of these poor reaction specimens, the full scope of injury could not be accurately ascertained. Seven control spot also died during the 24 h observation period. Two that were found dead at 4 h showed no obvious gross injury or subsequent histopathology. Five were dead at 24 h: two had alimentary canal necrosis and a heavy parasite burden; two had been tail-nipped by cage-mates; and one showed no discernible trauma. Three of the seven dead controls also had a poor stain reaction due to autolysis. The generally poor quality of the pinfish and spot that died and were either eviscerated or had a poor staining reaction precluded accurate injury assessment. Therefore, those specimens were excluded from further analysis.

Histological examination of specimens killed and immediately fixed at the end of the 24 h observation period revealed significant trauma in both species, particularly in those exposed to the strongest shockwaves. Injuries were observed to the kidney, swimbladder, liver and pancreas. For both species the most common shockwave-induced injury was hematuria (i.e.

damage to the tubules of the kidney). Others injuries included hemorrhage within the coelom, swimbladder hemorrhage, liver hemorrhage, coagulative liver necrosis and ruptured pancreas (Fig. 8). An additional trauma, alimentary canal necrosis, was frequently noted in both species. This condition was observed in exposed and control fish at all distances from the detonation site. It was noted in 44.8% of spot (25/65 exposed and 14/22 control) and 22.8% of pinfish (10/61 exposed and 8/18 control). Alimentary canal necrosis is unrelated to shockwave exposure. It is a known ailment of captive fish that have been fed a heavy diet of processed fish food (Mobin et al., 2000; Mobin et al., 2001). Coelomic hemorrhage was observed in two pinfish from the controls. A detailed account of the histopathology is provided in (Govoni et al., 2002).

The proportion of fish injured was highest nearest the blast and declined with increasing distance (Table 3). Spot injury was minimal below P_{\max} of about 250 kPa. Above 600 kPa, 100% of the spot were injured (Fig. 9), although, in one case 100% were injured at 280 kPa. Pinfish were more sensitive at lower P_{\max} , but, showed less and more variable sensitivity than spot as P_{\max} increased to about 900 kPa (Fig. 9). Spot injury was low at $I < 6$ Pa s, but, all spot were injured when $I > 10$ Pa s. Impulse induced injury to pinfish was highly variable (Fig. 10). Most of the injury to spot occurred at $EFD > 1 \text{ Jm}^{-2}$, but, again the response of pinfish was less marked or consistent (Fig. 11).

In an effort to reduce the observed variability in fish injury response to identify any general pattern that could be used to predict injury impact levels, the mean proportion injured was regressed on mean P_{\max} , I and EFD at each distance for each species (Figs. 12-17). No function relating mean lethal dose to spot with any shockwave parameter could be determined with the available data. The remaining empirical models were used to estimate SLD, LD and TID

at the 1%, 10%, and 50% levels over the range of available data (Table 4). Spot and pinfish were about equally sensitive to P_{\max} at the SLD_{10} level. Although the mean LD's for spot could not be modeled, they clearly experienced a greater mortality than pinfish at $P_{\max} > 550$ kPa (Figs. 12 and 15). Based on TID_{50} , spot were slightly more sensitive than pinfish to P_{\max} (Table 4). Pinfish, however, were about twice as sensitive as spot to I at all injury levels that could be assessed. Both species were nearly equally sensitive to EFD (Table 4). Unfortunately, our data did not permit an estimation of impact level in 6 of 9 cases for spot and in 4 of 9 cases for pinfish.

Impact of blasting to larval fishes in the Wilmington Harbor:

The shockwave pressure field associated with production blasts in the Wilmington Harbor exhibited typical exponential decay with distance from the detonation (Figs. 18-26). The impact radius for P_{\max} for spot and pinfish, for a specified injury level, was similar and varied little with depth (Table 5). The impact radii for I were the most extreme of any of the three shockwave parameters for both species. Due to their greater sensitivity, the impact radius for pinfish was 13-62 m farther, depending on depth, than that of spot (Table 5). The impact radius for I was also greater at the surface and near the river bottom. That was also the case for the EDF impact radius. The radii were similar for both species and intermediate between those obtained for P_{\max} and I (Table 5).

The volume of water surrounding a production blast for a specified injury level was estimated using the maximum impact radius at any depth for each shockwave parameter (i.e., P_{\max} , I and EFD) from Table 5. The outer boundaries of these volumes delineate where a specified injury level would occur (Table 6). Taking TID_{50} for I for each species (spot = 607,091

m^3 and pinfish = 817,003 m^3) as a conservative volume estimate, in conjunction with the mean concentrations of larval spot and pinfish in the lower river channel (see below), provides an estimate of the number of fish injured (and/or killed) per blast during the period of blasting when these species are present (i.e. December-January). The mean concentration of larval and recently metamorphosed spot in the Wilmington Harbor project area during their annual immigration period from 1976-1993 was 104 fish 1000 m^{-3} (s.d. 80). The maximum annual mean was 365 fish 1000 m^{-3} . Pinfish were considerably less abundant. The mean annual concentration of pinfish from 1988-1993 was 10 fish 1000 m^{-3} (s.d. 8) with a maximum annual mean concentration of 23 fish 1000 m^{-3} (Copeland et al., 1979; Carolina Power & Light, 1986;1994). Because of the greater abundance of spot, many more are predicted to be injured (Table 7).

The lower Cape Fear River is inhabited by many other species of larval fish in addition to spot and pinfish (see Settle and Fuss, 1997). The annual mean concentration of all species of fish larvae in the river, during August-January, from 1988-1993 was 1,584 fish 1000m^{-3} (s.d. 336) with a maximum mean of 2,014 fish 1000 m^{-3} (Carolina Power & Light, 1994). Assuming that those other species of fish that are present in the river during the blasting window have sensitivities on the order of those found in spot and pinfish, an estimate of the maximum number of larvae of all species injured per blast was about 1.1 million ($1,584 \text{ larvae } 1000\text{m}^{-3} \times 712,047 \text{ m}^3$); nearly 28% greater than the mean number killed daily due to entrainment at the Brunswick Steam Electric Plant cooling intake (Table 8). Thus, over the 5-7 year duration of the Wilmington Harbor project about 8.2×10^8 larvae and early juvenile fishes ($1,127,882 \text{ larvae blast}^{-1} \times 725$ blasts) could be injured and/or killed by blasting (Table 9).

Discussion

Our results show that late-larval and recently transformed juvenile spot and pinfish are vulnerable to underwater shockwaves produced by blasting. Fitch and Young (1948) reported that larval anchovies (Engraulidae) were killed by underwater shockwaves in California coastal waters and Nix and Chapman (1985) observed larval northern anchovy (*Engraulis mordax*) and smelt (Osmeridae) mortality associated with buried charge detonations with 25 ms delays. Unfortunately, no information on pressure field was provided with either observation. Kostyuchenko (1973) reported explosive shockwave injury to eggs and larvae of an engraulid (*Engraulis encrasicolus*) and carangid (*Trachurus mediterraneus*) at distances 10-20 m from the detonation. Survival of eggs after 24 h was about 58% at a range of 10 m and 98% at a range of 20 m. Although Kostyuchenko (1973) did not report any shockwave characteristics, based on the equation for predicting P_{\max} for TNT given in MacLennan and Simmonds (1992), P_{\max} at 10 m and at 20 m would have been about 1 MPa and 0.4 MPa. Injuries observed in exposed eggs included curling up of the embryo, collapsed membrane and displacement of the yolk. A limited number of observations made on newly hatched larvae, apparently lacking a swimbladder, noted trauma to the head and anterior intestine at these close ranges. Yelverton et al. (1975) estimated that the LD_{50} for larval guppy (*Lebistes reticulatus*) was 1.7 psi ms (11.7 kPa s). While we were unable to determine LD_{50} for spot and pinfish, our TID_{50} values were 8.9 Pa s and 5.3 Pa s, respectively. It appears that in general, larval fish, especially those with a swimbladder, are highly sensitive to shockwaves from underwater explosions.

In this study we examined the effect of underwater shockwaves on two physoclists (i.e. closed swimbladder). Other studies have shown that both physotomes (i.e. open swimbladder)

and physoclists are frequently killed by underwater explosions (Hubbs and Rehnitz, 1952; Tiller and Coker, 1955; Gaspin et al., 1976; Linton et al., 1985; Munday et al., 1986). Thus, the presence of an open or closed swimbladder appears to make little if any difference in a fish's vulnerability to injury from shockwaves (Gaspin, 1975; Wright, 1982).

Cronin (1948), Wiley and Wilson, (1975) and Gaspin et al. (1976) considered fish anatomy an important influence on sensitivity to injury. Thin-walled swimbladders are more vulnerable to injury than thick-walled swimbladders (Falk and Lawrence, 1973). The swimbladder in fishes with a loosely attached swimbladder typically ruptures along a single lengthwise split, whereas, in fishes with a more securely attached swimbladder, rupture usually occurs as one or more perforations along the ventro-lateral surface. The swimbladder of larval spot is weakly attached to the dorsal peritoneum (Govoni and Hoss, 2001) and Wiley et al. (1981) noted that the swimbladder of larger juvenile and adult spot usually ruptures along a single straight tear. We did not, however, observe swimbladder rupture in this study. In addition to the swimbladder, injuries to the kidney and liver are commonly observed in sciaenids (Cronin, 1948; Linton et al., 1985, Gaspin, 1975). We observed injuries to the swimbladder, liver, kidney and coelom of a sciaenid and a sparid (Fig. 8).

In nature, any injuries that persist for an extended period of time (order of days) are probably placing the fish at a serious disadvantage in capturing prey and avoiding predators. In this study liver necrosis and rupture of the pancreas were considered lethal. Combinations of less serious injuries (i.e., hemorrhage in the coelom, swimbladder, liver and hematuria) equivalent to Level 2 (see Table 2) are also considered lethal. In that most conservative sense, even sub-lethal injury likely results in mortality (Rosenthal and Alderdice, 1976). For example, stunned and

disoriented sand lance (*Ammodytidae*) were aggressively preyed upon by Atlantic mackerel (*Scomber scombrus*) immediately following a blast (Ross et al., 1985). Thus, our use of TID₅₀ to assess the cumulative impacts on larvae would appear justified (Tables 7-9).

Since the injurious effects of shockwaves varies with fish size, species, anatomy, orientation of the fish relative to the shockwave, type of explosive, depth of detonation, depth of the fish, water depth and bottom type, most shockwave parameters have generally been found to be a poor predictor of injury. Some of the variability in injury sustained by fishes at a given location observed in this study (Table 3 and Figs. 5-7) may be due to shielding effects by other fish or the acoustic sensors (Gaspin et al., 1976). Another potential confounding factor in relating shockwave parameters to fish injury is the effect of testing un-acclimatized fish at depth (Gaspin et al., 1976). The coelomic hemorrhage observed in two control pinfish may have been caused by subjecting those un-acclimatized fish to relatively sudden pressure changes.

MacLennan and Simmonds (1992) reviewed existing predictive models, which relate I (Yelverton et al., 1975), swimbladder oscillation (Goertner (1978; Wiley et al., 1981) or EFD (Baxter, 1985) to fish injury and found none were entirely satisfactory. The Goertner (1978) and Wiley et al. (1981) approach does a satisfactory job at predicting injury to fish at any depth and range, but, in addition to requiring data on the shockwave pressure field and fish concentration within the affected volume, it requires species-specific data on swimbladder radius. It would seem then to be impractical for field applications. Impulse appears to be a reliable shockwave parameter for predicting injury in the upper 10 m of the water column and it is over that region, where the impulse damage parameter model (Yelverton et al., 1975) and the swimbladder oscillation parameter model (Goertner, 1978; Wiley et al., 1981) are essentially equivalent

(Wiley et al., 1981). Yelverton et al.,'s (1975) impulse model also performs poorly when the bottom is rocky (Wright, 1982). Munday et al., (1986) examined fish kill associated with time delayed linear explosive charges buried in conglomerate rock and found that P_{\max} and I were greatly reduced below that predicted for mid-water detonations. The highest impulse was observed near the air-surface interface and near the bottom. The Wilmington Harbor blasting data support that observation (Figs. 18-26). Thus the predictions using the model of Yelverton et al. (1975) are not valid for buried charges and result in poor predictions of injury to fishes. Baxter's (1985) EFD model is also able to predict injury at depths greater than a few meters from the surface and Sakaguchi et al., (1976) considered EFD a better predictor of fish injury than P_{\max} . The rate of change in P_{\max} is the basic principle in EFD models. Apparently, neither P, I or EFD alone is a reliable predictor of injury under all conditions (Gaspin, 1975). Our results support this view as our predicted impact radius (volume) varied widely depending on which shockwave parameter was considered (Tables 5 and 6).

One shortcoming of models incorporating I or EFD is the fact that time scale of integration usually does not include the period of arrival of all incident shockwaves, which are likely important in fish injury. The rapid decompression of gas in the swimbladder to a level below ambient due arrival of the rarefaction wave, following sudden compression is most likely the direct cause of mortal injury (Hubbs and Rehnitzer, 1952). Most evidence indicates that it is the rapid decompression upon arrival of the rarefaction wave that is the principle cause of injury (Wright, 1982). Below some unknown peak over-pressure extrema, fish are relatively more resistant to sudden compressive forces exerted by shockwaves than to sudden pressure drops such as experienced upon arrival of the rarefaction. In those cases, P_{\min} and it's time of arrival

following P_{\max} would seem to be important when considering the relationship between underwater explosions and fish injury (MacIennan and Simmonds, 1992). Since the temporal scale of integration of both I and EFD (i.e., 3-5 decay constants) includes only the positive pressure component of the primary shockwave, the potentially injurious negative pressure associated with arrival of the surface-reflected wave are not accounted for. It seems likely that a new parameter relating fish injury to P_{\min} and the rate of pressure change from P_{\max} to P_{\min} should be considered. This consideration is implicit in the aforementioned swimbladder oscillation model (Goertner, 1978; Wiley et al., 1981) as swimbladder rupture is dependent upon the time of arrival of P_{\min} during the oscillatory cycle of the bladder after P_{\max} . It predicts rupture when resonance occurs (i.e., P_{\min} is coincident with bladder compression).

Our use of simple empirical models based on P_{\max} , I and EFD, were useful for estimating injury level in the present study and should have applicability for similar engineering projects involving underwater blasting. However, these models do not further the quest to find a more robust general model for predicting fish injury from underwater shockwaves. Theoretical considerations imply that the response (i.e. proportion injured) curve for a dose (i.e. some shockwave characteristic) should have a threshold below which little or no injury would occur and above which injury would rise rapidly to an asymptote at or near 100% injury: the familiar dose-response curve. There is a hint of such a response in spot to all three parameters (Figs. 9-11). Gaspin (1975) also noted a tendency toward an "all or nothing" mortality response in spot and Wiley et al. (1981) was able to fit a typical dose-response curve to juvenile and adult spot injury (Level 2 and 3) and a calculated swimbladder oscillation parameter.

The use of explosives to aid in the removal of rock in the navigation channel of the upper

Wilmington Harbor project in the Cape Fear River will undoubtedly result in injury and mortality of some organisms including larval fishes. Our results suggest that nearly 8.2×10^8 larvae could be injured or killed over the duration of the project (725 blasts over 5-7 years). However, considering that the tidal prism of the Cape Fear River is on the order of $1.6 \times 10^9 \text{ m}^3$ (NOAA, 1985), up to 5.1×10^9 larvae ($1,584 \text{ larvae } 1000 \text{ m}^{-3} \times 1.6 \times 10^9 \text{ m}^3 \text{ tide}^{-1} \times 2 \text{ tides day}^{-1}$) could pass through the production site per day during an annual blasting window. Given that, the total number of larvae injured and/or killed (i.e., 8.2×10^8) over the duration of the project (725 blasts) would represent 3.2% (5 year) or 2.3% (7 year) of the total number of larvae passing through the project area during all the August-January blasting windows combined over the life of the project (i.e., 5-7 years). It seems reasonable to conclude that such a low level of impact is unlikely to affect the local population.

The current blasting monitoring plan calls for P_{max} to average 483 kPa and not exceed 827 kPa at a distance of 43 m from the detonation site (USACE, 2000a). Most larvae exposed to those peak pressures will be killed. Based on our analysis and conclusion of no population-level impact, it is recommended that the near-surface impulse should not exceed 7 Pa s (i.e., the mean TID_{50} for I for spot and pinfish) at a distance of 210 m (i.e., the radius where I is predicted to be 7 Pa s based on production test blasting using the relation given in Figure 21).

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Table 1. Experimental design and number of replicates. Each replicate and control consisted of a bag containing 15-30 fish.

Species	Distance from detonation site					
	3.6 m		7.5 m		17 m	
	Treatment	Control	Treatment	Control	Treatment	Control
<i>Lagodon rhomboides</i>	3	1	3	1	3	1
<i>Leiostomus xanthurus</i>	3	1	3	1	3	1

Table 2. Scaled injury criteria for fishes exposed to underwater explosions. Damage level of 2 or greater is lethal in nature (after Hubbs et al., 1960).

Damage Level	Damage Criteria
(0)	No damage
(1)	Only light hemorrhaging, principally in the tissues covering the kidney
(2)	Swimbladder intact, but with light hemorrhaging throughout the body cavity, with some damage to the kidney
(3)	No external indication of damage, but with the swimbladder usually burst. Hemorrhaging and organ disruption less extreme than in (4) and (5), but with gross damage to the kidney.
(4)	Incomplete break-through of the body wall, but with bleeding about the anus. The swimladder is almost invariably broken and other organs damaged as noted in (5).
(5)	Rupture of the body cavity. The break is usually a slit just to the side of the midventral line. Associated with the severe damage is a burst gasbladder and gross damage to other internal organs. The abdominal contents are often completely lost or homogenized.

Table 3. The number (n) and standard length (SL) of early juvenile spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboides*) maintained in plastic test bags and used in the field experiment to assess impact of exposure to underwater explosions. Seawater temperature (T) and dissolved oxygen (D.O.) in the bags were monitored over the duration of the experiment Handling time and submergence time were recorded for each bag. Peak pressure (P_{max}), minimum pressure (P_{min}), impulse (I) and energy flux density (EFD) resulting from each of the blast-induced underwater shock waves are provided along with observed mortality and injury at three time intervals, the number of specimens examined during histological injury assessment and the proportion of fish injured. NA indicates not applicable and identifies the control bags.

Species	Bag	n	SL (mm)	T °C	D.O. mg l ⁻¹	Handling ¹	Submergence ²	Blast	Distance (m)	P _{max} (kPa)	P _{min} (kPa)	I (Pa s)	EFD (J m ⁻²)	Observed			Histology specimens	Proportion injured ⁴	
			± (s.d.)	± (s.d.)	± (s.d.)	time (min.)	time (min.)							mortality/injury ³	0 hr	4 hr		24 hr	sub-lethal/
Leiostomus xanthurus	SL1R1	21	19.1 (1.7)	13.71 (0.94)	6.62 (0.33)	56	14	16	3.6	691.5	-60.6	12.080	3.642	1/0	0/0	0/3	10	0.20	0.80
	SL1R2	18	18.9 (0.9)	13.56 (0.56)	6.12 (0.43)	48	9	17	3.6	742.6	-203.4	10.859	2.238	0/0	0/0	2/3	11	0.22	0.78
	SL1R3	16	20.1 (1.4)	13.37 (0.39)	6.00 (0.32)	42	7	18	3.6	277.9	-49.6	10.046	1.096	0/0	0/0	1/2	9	0.37	0.63
	SL1C	25	19.9 (1.3)	13.39 (0.71)	5.60 (0.52)	51	8	NA	3.6	NA	NA	NA	NA	0/2*	1/1*	0/0	7	0	0
	SL2R3	20	18.8 (1.8)	13.80 (0.54)	5.57 (0.57)	62	10	19	7.5	216.5	-77.9	3.227	0.211	0/0	0/0	3/0	9	0	0
	SL2R2	21	19.1 (1.7)	13.93 (0.40)	5.92 (0.63)	58	9	20	7.5	146.9	-48.2	4.033	0.182	0/1	0/1	3/0	9	0.17	0
	SL2C	16	18.4 (1.2)	13.83 (0.41)	6.10 (0.13)	63	8	NA	7.5	NA	NA	NA	NA	0/2*	1/0	3/0	10	0	0
	SL2R1	20	19.8 (1.7)	14.21 (0.01)	6.15 (0.07)	99	29	21	7.5	290.3	-51.7	5.723	0.505	0/0	0/0	1/0	7	0.17	0
	SL3R1	23	18.5 (1.6)	13.97 (0.14)	6.24 (0.31)	84	55	22	17.0	123.4	-64.4	1.917	0.106	0/1*	1/0	4/0	11	0.14	0
	SL3R2	16	19.7 (1.9)	14.10 (0.40)	6.22 (0.18)	48	7	23	17.0	79.3	-32.4	2.075	0.058	0/0	0/0	0/0	6	0	0
	SL3R3	20	18.0 (1.4)	14.19 (0.30)	6.67 (0.78)	58	9	24	17.0	116.5	-73.1	2.151	0.108	0/2*	0/1	4/0	10	0	0
SL3C	16	18.5 (1.3)	14.09 (0.26)	6.22 (0.14)	31	8	NA	17.0	NA	NA	NA	NA	0/1	0/1	2/0	8	0	0	
Lagodon rhomboides	PL1R1	23	17.1 (1.2)	13.35 (0.45)	5.76 (0.51)	57	9	16	3.6	557.8	-42.7	2.682	1.311	0/1	1/0	0/0	7	0.50	0.50
	PL1R2	23	15.9 (1.1)	13.55 (0.29)	5.93 (0.46)	36	5	17	3.6	700.5	-108.3	9.563	2.594	0/0	0/0	1/0	8	0.57	0.14
	PL1R3	19	16.8 (1.0)	13.39 (0.57)	6.05 (0.60)	41	5	18	3.6	866.0	-89.6	6.971	2.582	0/1	0/0	0/3	9	0	0.33
	PL1C	21	17.1 (2.0)	13.68 (0.35)	5.60 (0.58)	52	6	NA	3.6	NA	NA	NA	NA	0/0	0/0	0/0	6	0	0
	PL2R3	19	16.8 (1.2)	13.68 (0.35)	5.64 (0.65)	61	5	19	7.5	297.2	-151.6	3.854	0.585	0/0	0/0	2/0	8	0.33	0.17
	PL2R2	25	17.0 (1.2)	13.90 (0.36)	5.77 (0.56)	57	4	20	7.5	119.3	-28.9	1.855	0.073	0/0	0/0	1/0	7	0.14	0
	PL2C	22	17.1 (1.2)	13.80 (0.35)	5.94 (0.47)	63	5	NA	7.5	NA	NA	NA	NA	0/0	0/0	0/0	6	0.17	0
	PL2R1	30	16.7 (1.4)	13.93 (0.38)	5.97 (0.18)	98	25	21	7.5	317.8	-68.8	5.102	0.614	0/0	0/0	0/0	6	0	0
	PL3R1	20	16.7 (1.3)	13.91 (0.30)	5.84 (0.82)	84	53	22	17.0	118.6	-60.0	2.482	0.126	0/0	0/0	0/0	6	0	0
	PL3R2	15	16.8 (0.9)	14.07 (0.35)	6.23 (0.18)	48	5	23	17.0	111.7	-46.2	2.220	0.125	0/0	0/0	0/1	7	0.14	0
	PL3R3	16	17.0 (1.0)	14.15 (0.29)	5.76 (0.80)	58	5	24	17.0	113.8	-89.6	2.255	0.103	0/0	0/0	0/0	6	0	0
	PL3C	18	17.2 (1.3)	14.03 (0.45)	6.07 (0.17)	33	7	NA	17.0	NA	NA	NA	NA	0/0	0/0	0/0	6	0.17	0

1. Handling time refers to the total time elapsed from removal of bags from laboratory water bath to the field until return to the water bath.

2. Submergence time refers to the total elapsed time the bag was held at a depth of 2 m.

3. Injured fish exhibited aberrant behavior (e.g. erratic swimming, disorientation).

4. Proportion injured as assessed by histology. Lethal injuries include ruptured pancreas and necrosis of the liver. Possible sub-lethal injuries include coelomic hemorrhage, hematuria, swimbladder hemorrhage, and liver hemorrhage in the absence of severe trauma just noted.

* indicates that these fish appeared injured, were removed alive and examined under a stereo dissecting microscope for signs of injury, none being readily observed the fish were immediately returned to the bag.

Table 4. Estimated sub-lethal dose (SLD₅₀, SLD₁₀, SLD₁), lethal dose (LD₅₀, LD₁₀, LD₁) and total injury dose (TID₅₀, TID₁₀, TID₁) for early juvenile *Leiostomus xanthurus* and *Lagodon rhomboides* and associated shockwave parameters resulting from underwater high-velocity explosives. n.d. indicates insufficient data.

Species	Level of impact	<u>Peak pressure</u>	<u>Impulse</u> ¹	<u>Energy flux density</u> ²
		kPa	Pa s	J m ⁻²
<i>Leiostomus xanthurus</i>	SLD ₅₀	n.d.	n.d.	n.d.
	SLD ₁₀	210	4.071	0.440
	SLD ₁	n.d.	n.d.	n.d.
	LD ₅₀	n.d.	n.d.	n.d.
	LD ₁₀	n.d.	n.d.	n.d.
	LD ₁	n.d.	n.d.	n.d.
	TID ₅₀	461	8.910	1.168
	TID ₁₀	210	4.090	0.240
	TID ₁	n.d.	n.d.	n.d.
<i>Lagodon rhomboides</i>	SLD ₅₀	n.d.	n.d.	n.d.
	SLD ₁₀	175	2.911	0.256
	SLD ₁	n.d.	n.d.	n.d.
	LD ₅₀	n.d.	n.d.	n.d.
	LD ₁₀	307	3.779	0.712
	LD ₁	142	2.662	0.149
	TID ₅₀	532	5.286	1.483
	TID ₁₀	148	2.718	0.167
	TID ₁	n.d.	n.d.	n.d.

1. Impulse was measured from time-of-arrival of the shockwave to 75 ms afterwards. This interval represents approximately three time decay constants and accounts for 95% of the total impulse.

2. Energy flux density was derived over the same interval as impulse.

Table 5. Estimated impact radius (m) at depth for three shockwave parameters resulting from a production blast in the Wilmington Harbor, NC. Injury level for larval and early juvenile *Leiostomus xanthurus* and *Lagodon rhomboides* are sub-lethal dose (SLD₅₀, SLD₁₀, SLD₁), lethal dose (LD₅₀, LD₁₀, LD₁) and total injury dose (TID₅₀, TID₁₀, TID₁). Depth are near-surface¹ (S), mid-depth² (M) and near-bottom³ (B). n.d. indicates insufficient data.

Species	Level of impact	Peak pressure			Impulse			Energy flux		
		S	M	B	S	M	B	S	M	B
<i>Leiostomus xanthurus</i>	SLD ₅₀		n.d.			n.d.			n.d.	
	SLD ₁₀	53	54	49	277	192	233	138	99	105
	SLD ₁		n.d.			n.d.			n.d.	
	LD ₅₀		n.d.			n.d.			n.d.	
	LD ₁₀		n.d.			n.d.			n.d.	
	LD ₁		n.d.			n.d.			n.d.	
	TID ₅₀	34	37	32	177	140	168	99	77	79
	TID ₁₀	53	54	49	277	192	233	170	115	126
	TID ₁		n.d.			n.d.			n.d.	
	SLD ₅₀		n.d.			n.d.			n.d.	
	SLD ₁₀	58	59	53	335	220	269	166	113	124
	SLD ₁		n.d.			n.d.			n.d.	
<i>Lagodon rhomboides</i>	LD ₅₀		n.d.			n.d.			n.d.	
	LD ₁₀	43	45	40	289	198	241	117	87	91
	LD ₁	65	66	60	352	228	279	200	129	145
	TID ₅₀	32	34	29	239	173	209	91	73	73
	TID ₁₀		63	64	59	348	226	277	192	126
	141									
	TID ₁		n.d.			n.d.			n.d.	

1. Nominal depth is 0.9 m.

2. Nominal depth is 4.6 m.

3. Nominal depth is 9.1 m.

Table 6. Estimated volume of water (m³) impacted per production blast in the Wilmington Harbor, NC for a specified level of injury to larval and early juvenile *Leiostomus xanthurus* and *Lagodon rhomboides*. Impact levels are sub-lethal dose (SLD₅₀, SLD₁₀, SLD₁), lethal dose (LD₅₀, LD₁₀, LD₁) and total injury dose (TID₅₀, TID₁₀, TID₁). n.d. indicates insufficient data.

Species	Level of impact	<u>Peak pressure</u> Volume (m ³) impacted	<u>Impulse</u> Volume (m ³) impacted	<u>Energy flux</u> Volume (m ³) impacted
<i>Leiostomus xanthurus</i>	SLD ₅₀	n.d.	n.d.	n.d.
	SLD ₁₀	126,264	947,911	581,732
	SLD ₁	n.d.	n.d.	n.d.
	LD ₅₀	n.d.	n.d.	n.d.
	LD ₁₀	n.d.	n.d.	n.d.
	LD ₁	n.d.	n.d.	n.d.
	TID ₅₀	57,422	607,091	339,107
	TID ₁₀	125,655	945,425	432,015
	TID ₁	n.d.	n.d.	n.d.
<i>Lagodon rhomboides</i>	SLD ₅₀	n.d.	n.d.	n.d.
	SLD ₁₀	150,036	1,147,139	569,245
	SLD ₁	n.d.	n.d.	n.d.
	LD ₅₀	n.d.	n.d.	n.d.
	LD ₁₀	86,010	988,980	591,034
	LD ₁	154,786	1,206,975	684,594
	TID ₅₀	49,852	817,003	358,625
	TID ₁₀	177,235	1,192,804	658,718
	TID ₁	n.d.	n.d.	n.d.

Table 7. Estimated mean and maximum number of larval and early juvenile *Leiostomus xanthurus* and *Lagodon rhomboides* injured per production blast (Dec.-Jan.) based on TID₅₀ for three shockwave parameters. Estimates include both sub-lethal and lethal injury.

Species	<u>Peak pressure</u>		<u>Impulse</u>		<u>Energy flux</u>	
	larvae injured blast ⁻¹ mean	maximum	larvae injured blast ⁻¹ mean	maximum	larvae injured blast ⁻¹ mean	maximum
<i>Leiostomus xanthurus</i>	5,792	20,959	63,137	221,588	35,267	123,774
<i>Lagodon rhomboides</i>	499	1,147	8,170	18,791	3,586	8,248

Table 8. Estimated mean (s.d.) number of larval fishes killed per day by entrainment at the Brunswick Steam Electric Plant cooling intake and larval fishes injured per blast in the Wilmington Harbor. Estimates for three shockwave parameters include both sub-lethal and lethal injury.

<u>Entrainment</u>		<u>Peak pressure</u>		<u>Impulse</u>		<u>Energy flux</u>	
larvae killed d ⁻¹		larvae injured blast ⁻¹		larvae injured blast ⁻¹		larvae injured blast ⁻¹	
mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
814,667	696,685	84,961	18,022	1,127,882	239,248	552,603	117,219

Entrainment data are from 1993 and larval fish data are from 1988-1993. Carolina Power & Light Company. 1994. Brunswick Steam electric Plant 1993 Biological Monitoring Report, Environmental Services Section, Southport, NC.

Blast induced injury is based on the mean larval fish concentration data from CP&L and the impacted volume computed from the average TID₅₀ for *Leiostomus xanthurus* and *Lagodon rhomboides* obtained in the present study.

Table 9. Estimated number of larval fishes injured due to blasting over the duration of the Wilmington Harbor project. Estimates for three shockwave parameters include both sub-lethal and lethal injury.

<u>Peak pressure</u> larvae injured	<u>Impulse</u> larvae injured	<u>Energy flux</u> larvae injured
61,596,371	817,714,421	400,637,240

The total blast induced injury is based on the mean larval fish concentration (1988-1993) from Carolina Power & Light Company. 1994. Brunswick Steam electric Plant 1993 Biological Monitoring Report, Environmental Services Section, Southport, NC., and the impacted volume computed from the average TID₅₀ for *Leiostomus xanthurus* and *Lagodon rhomboides* obtained in the present study and assumes 725 blasts over the duration of the project.

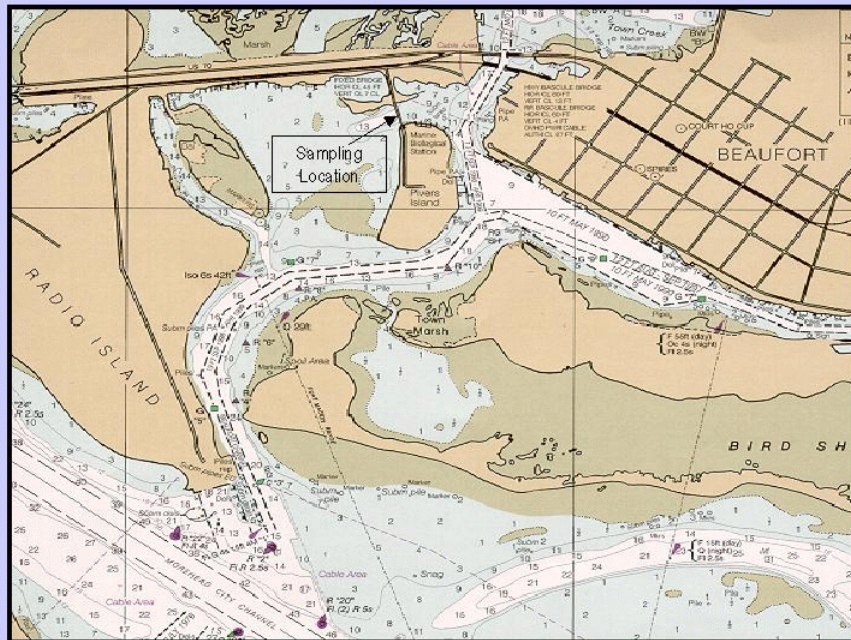
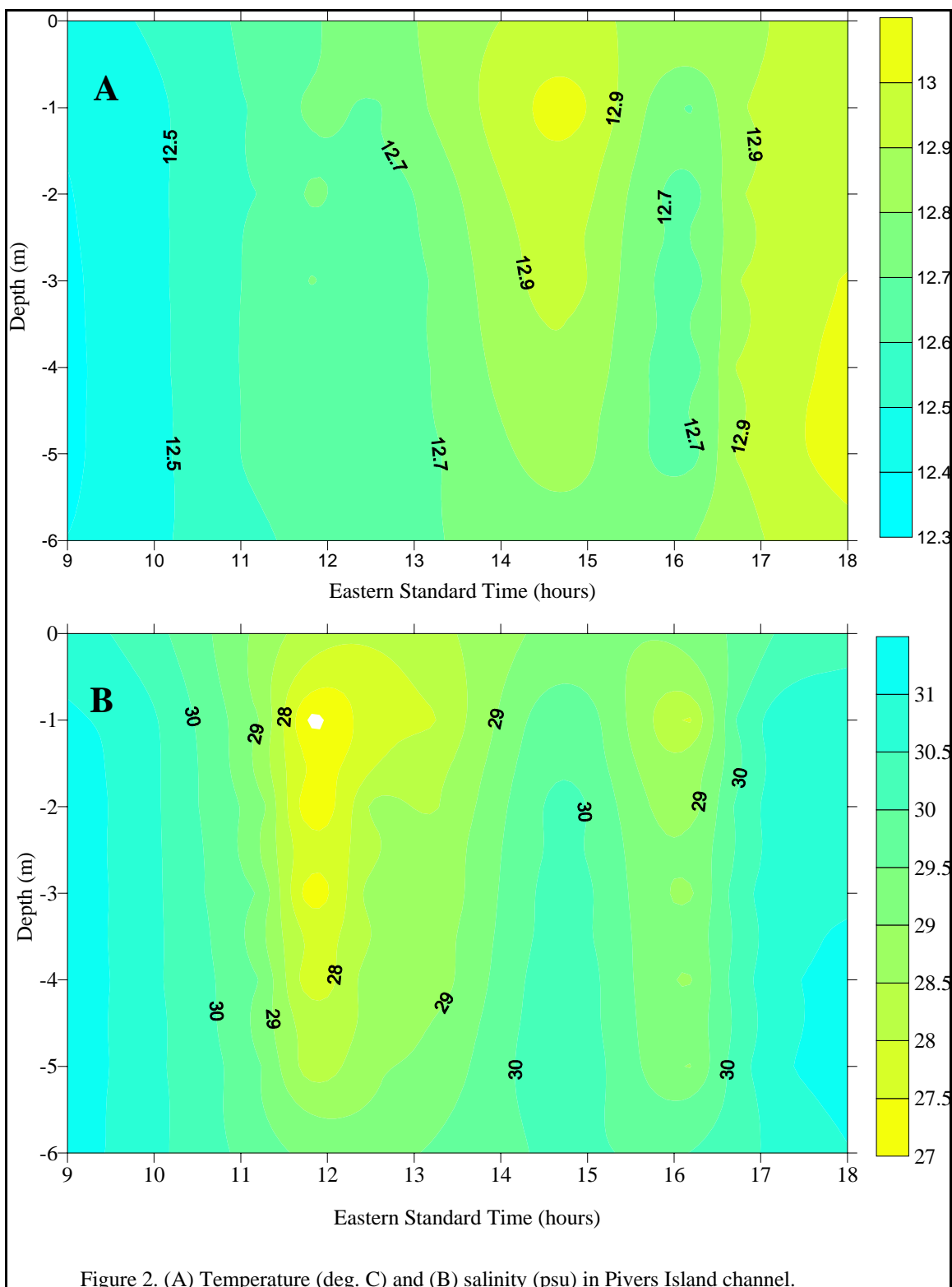
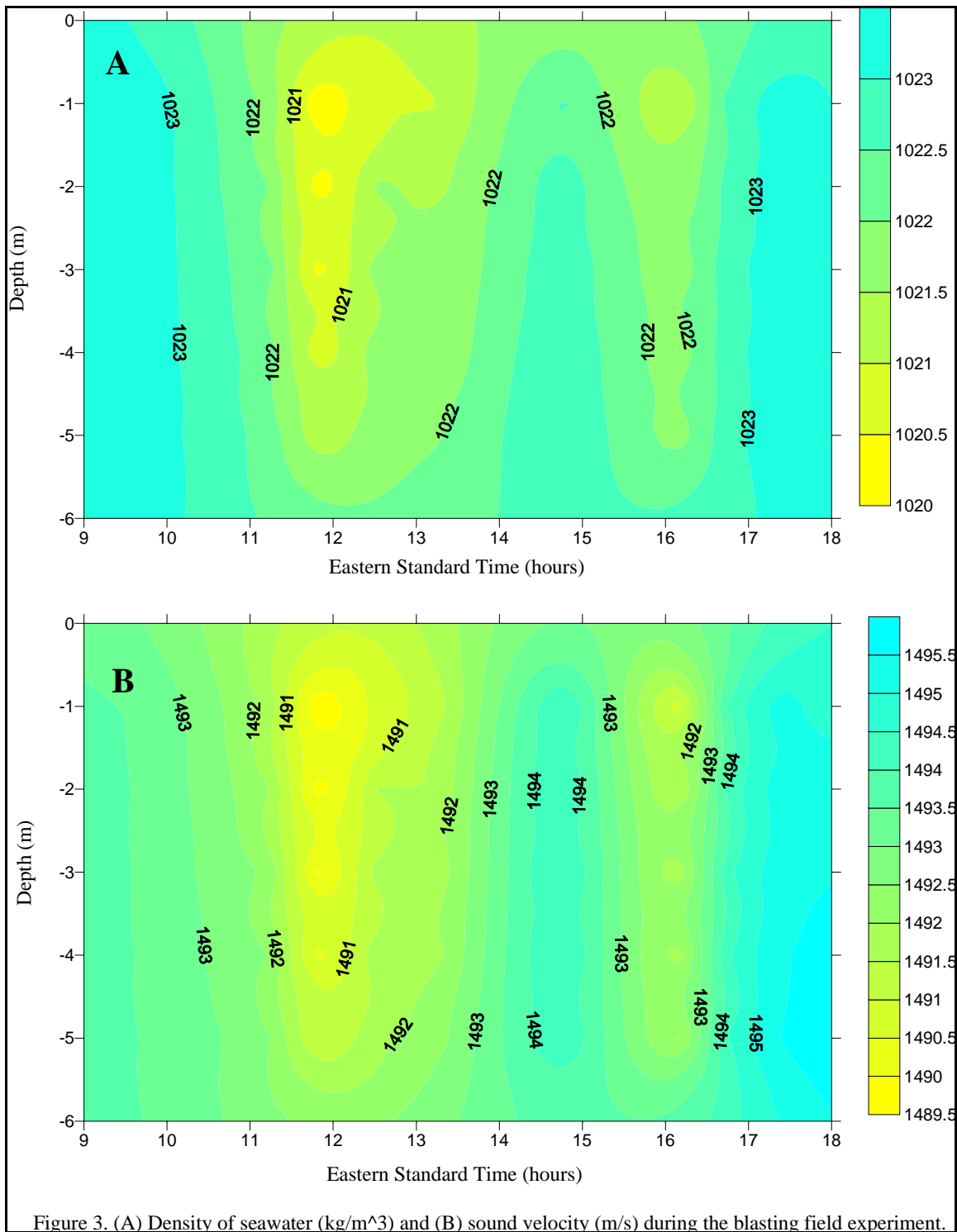


Figure 1. Location of study area.





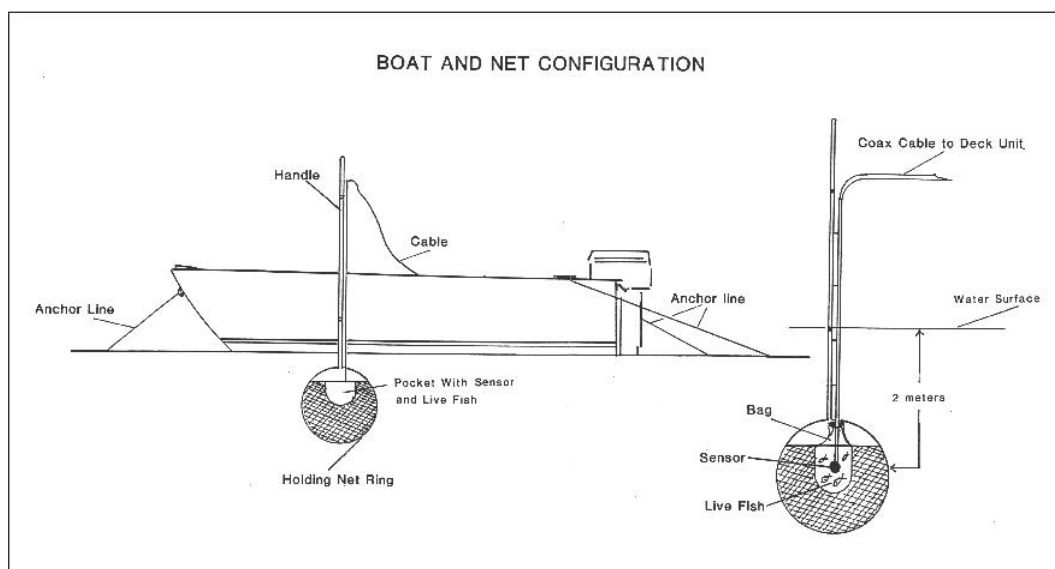
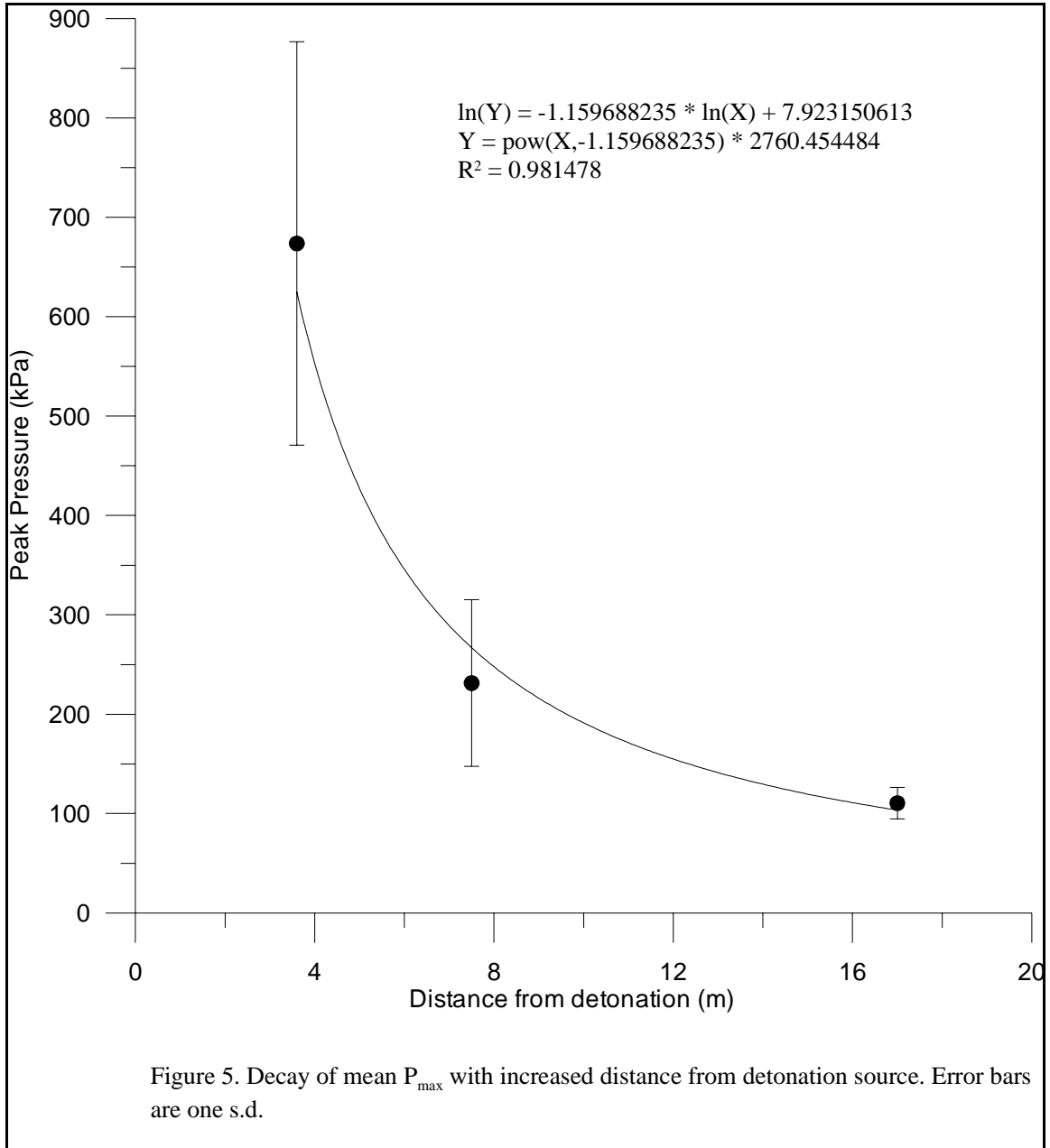
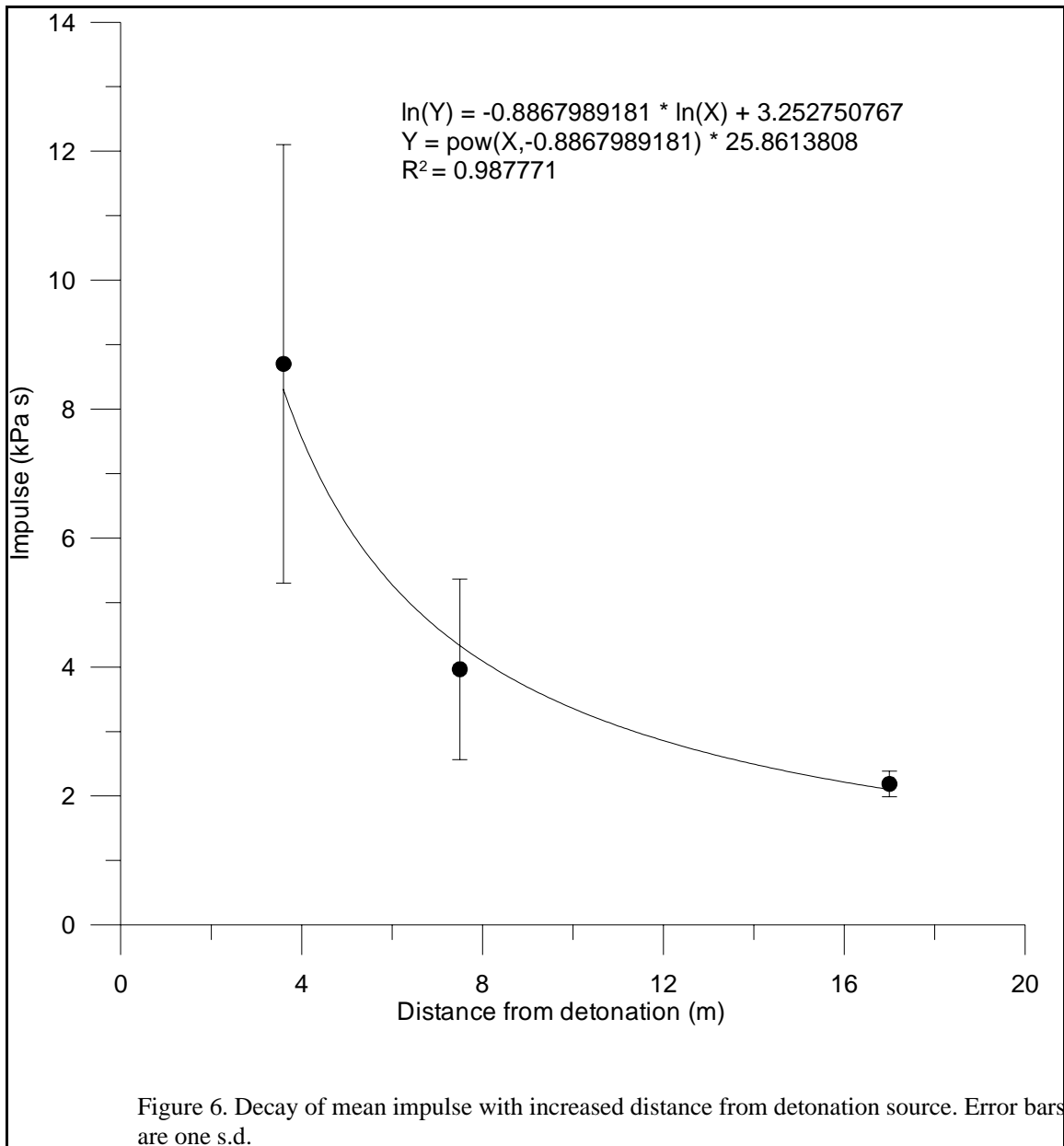
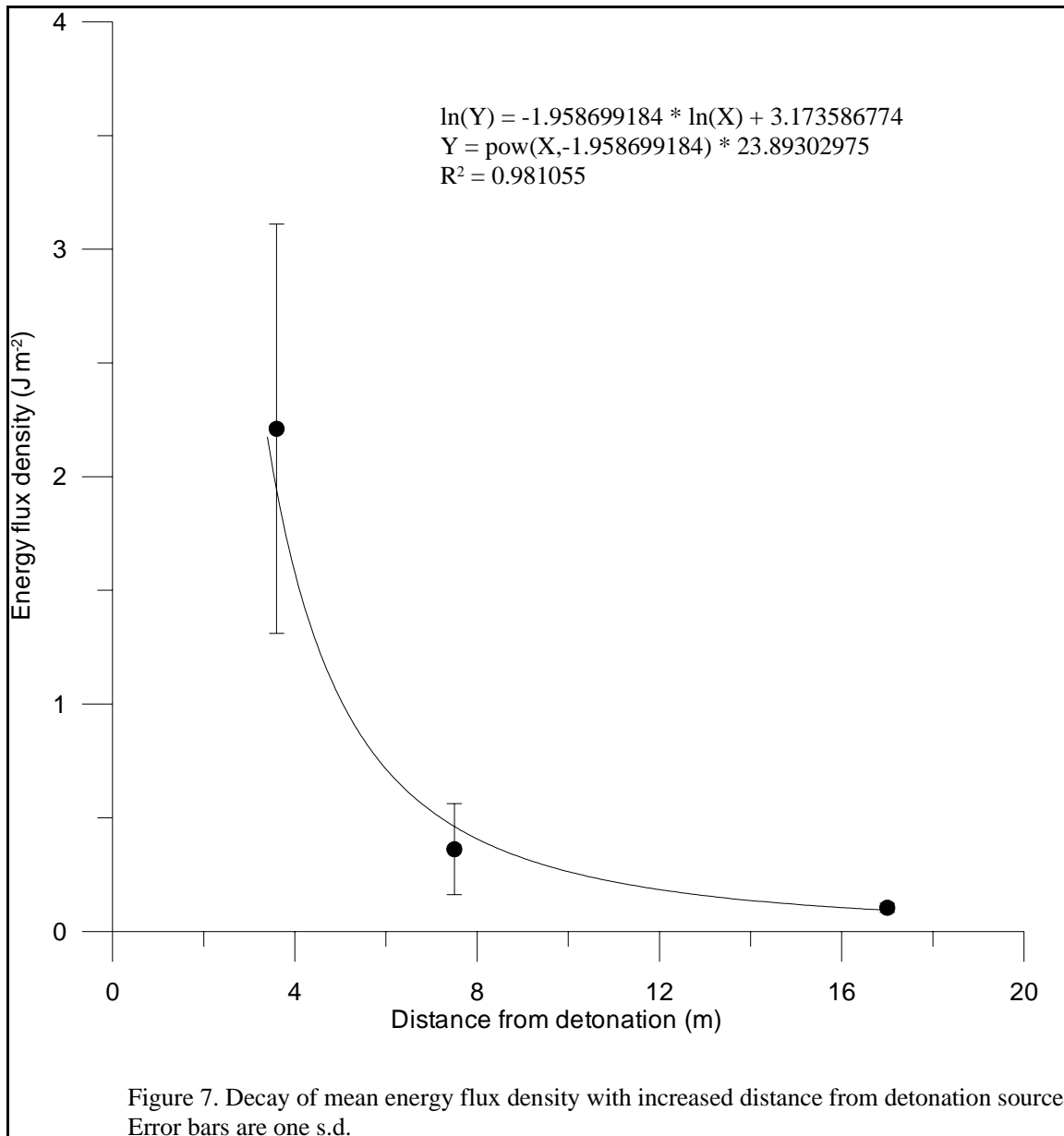


Figure 4. Schematic of field exposure rigging.







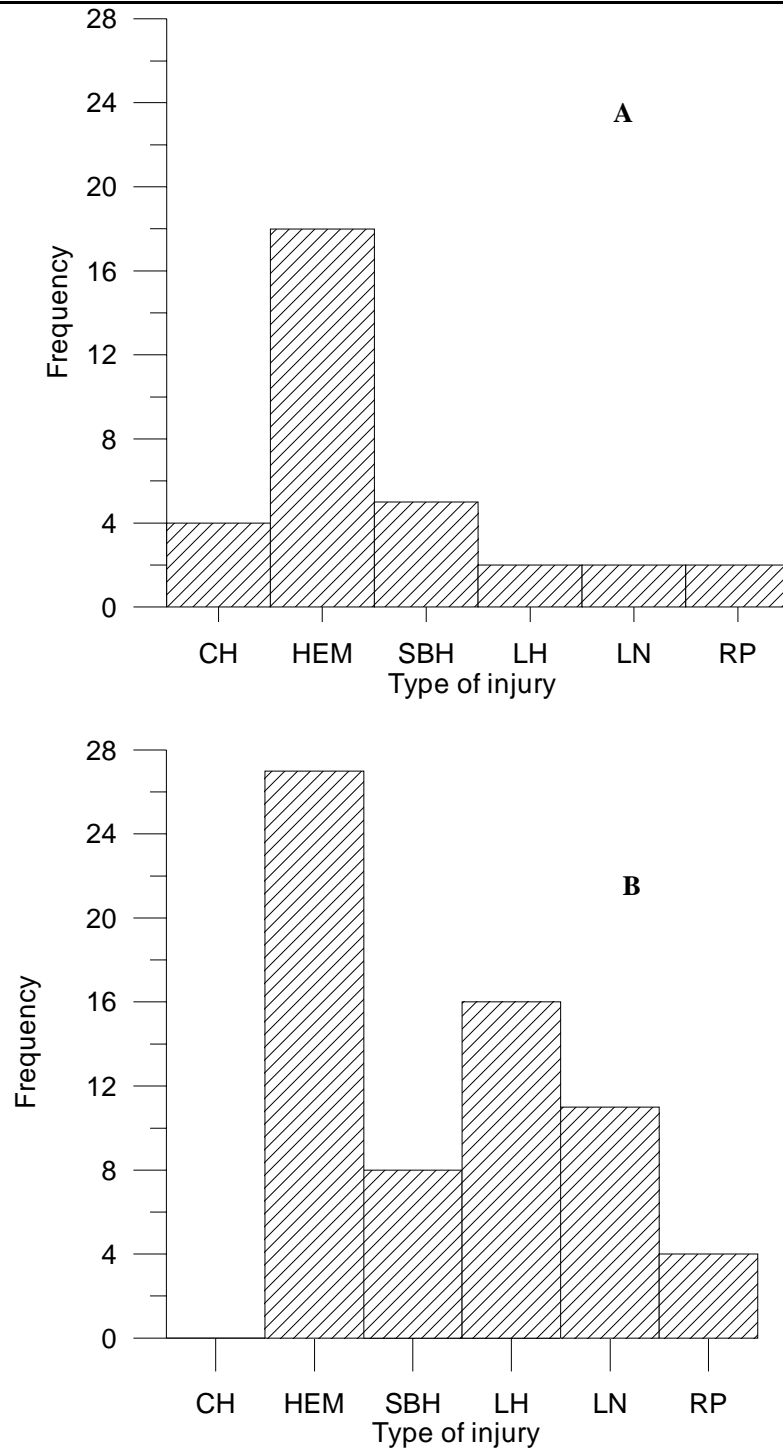
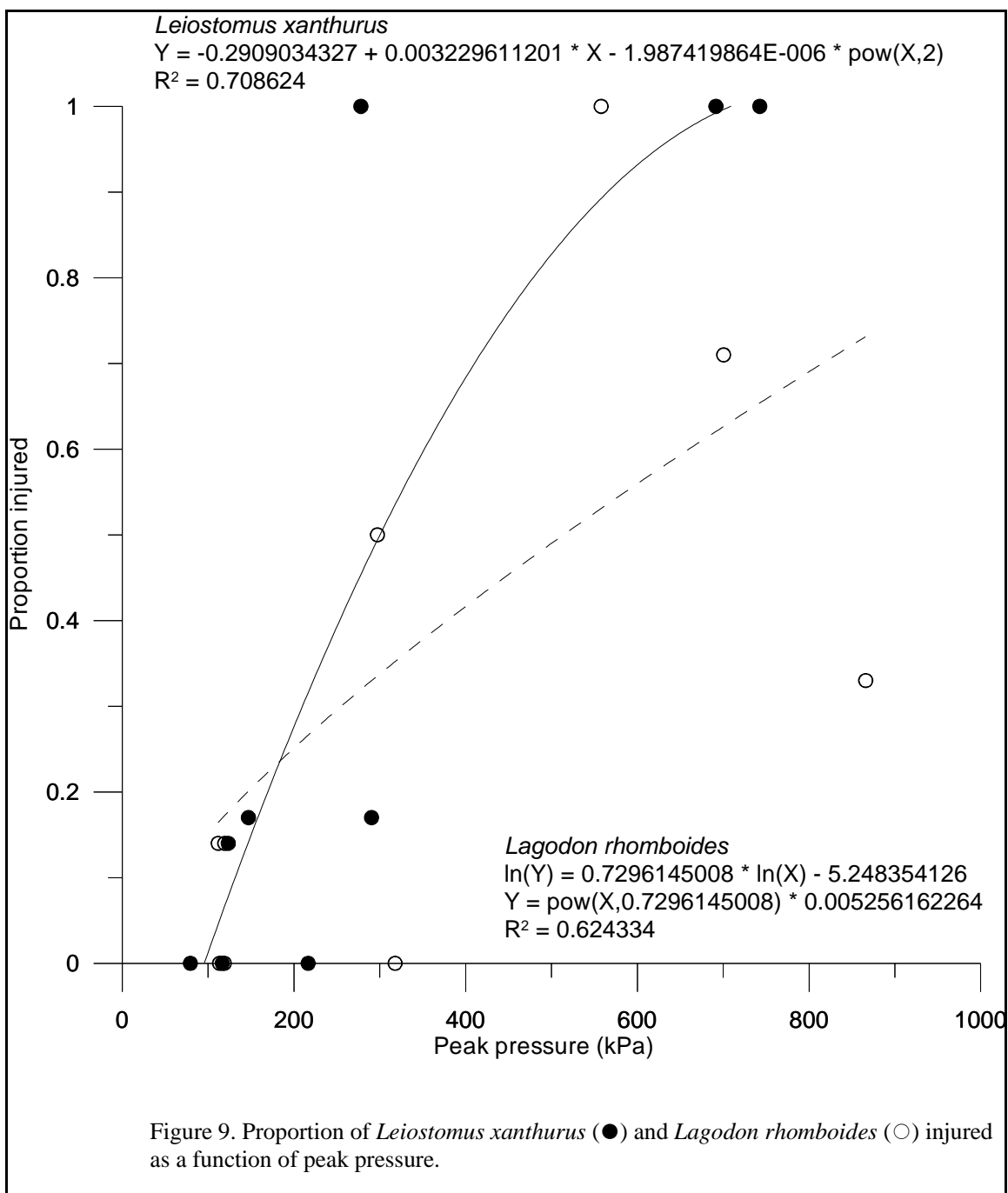
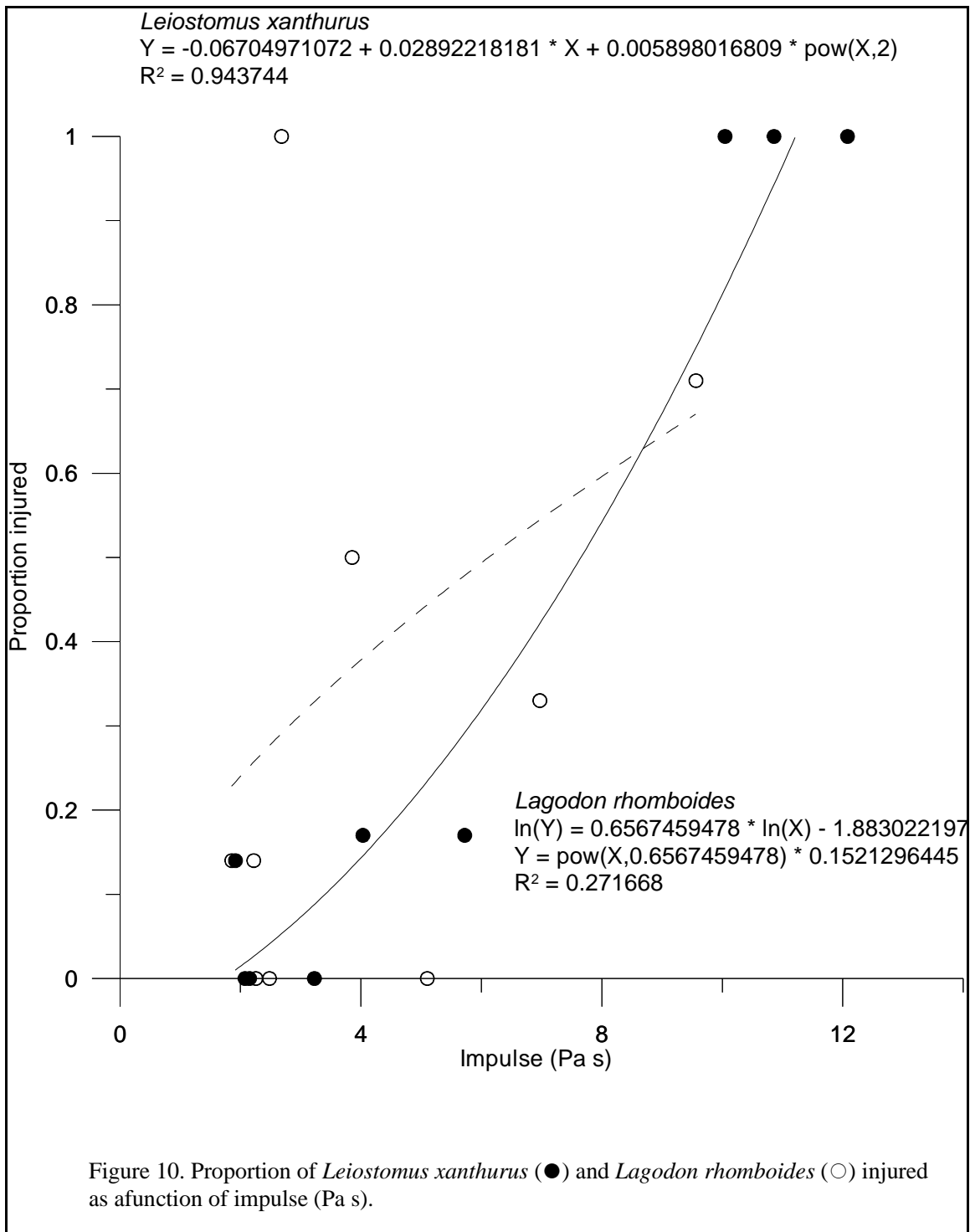
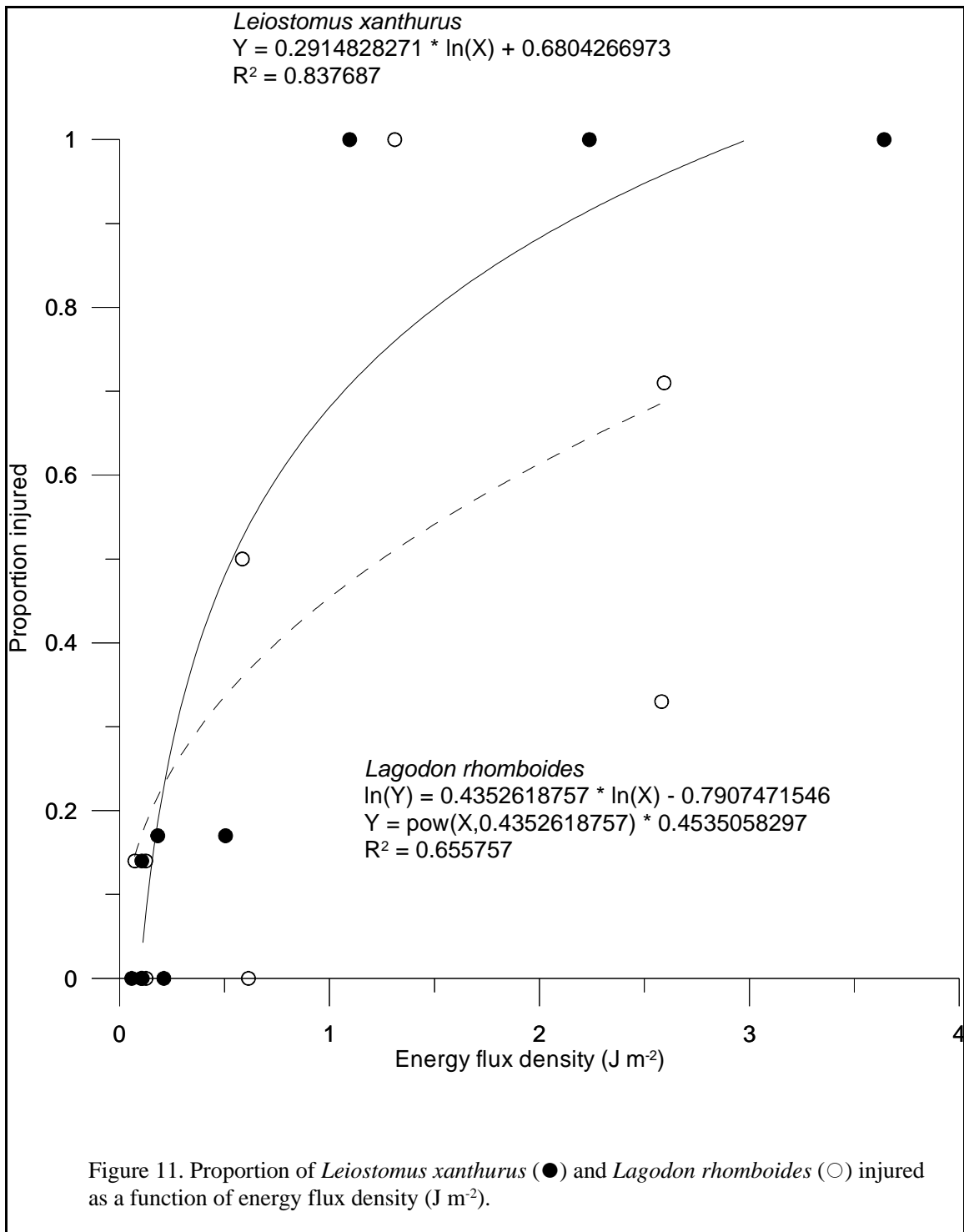
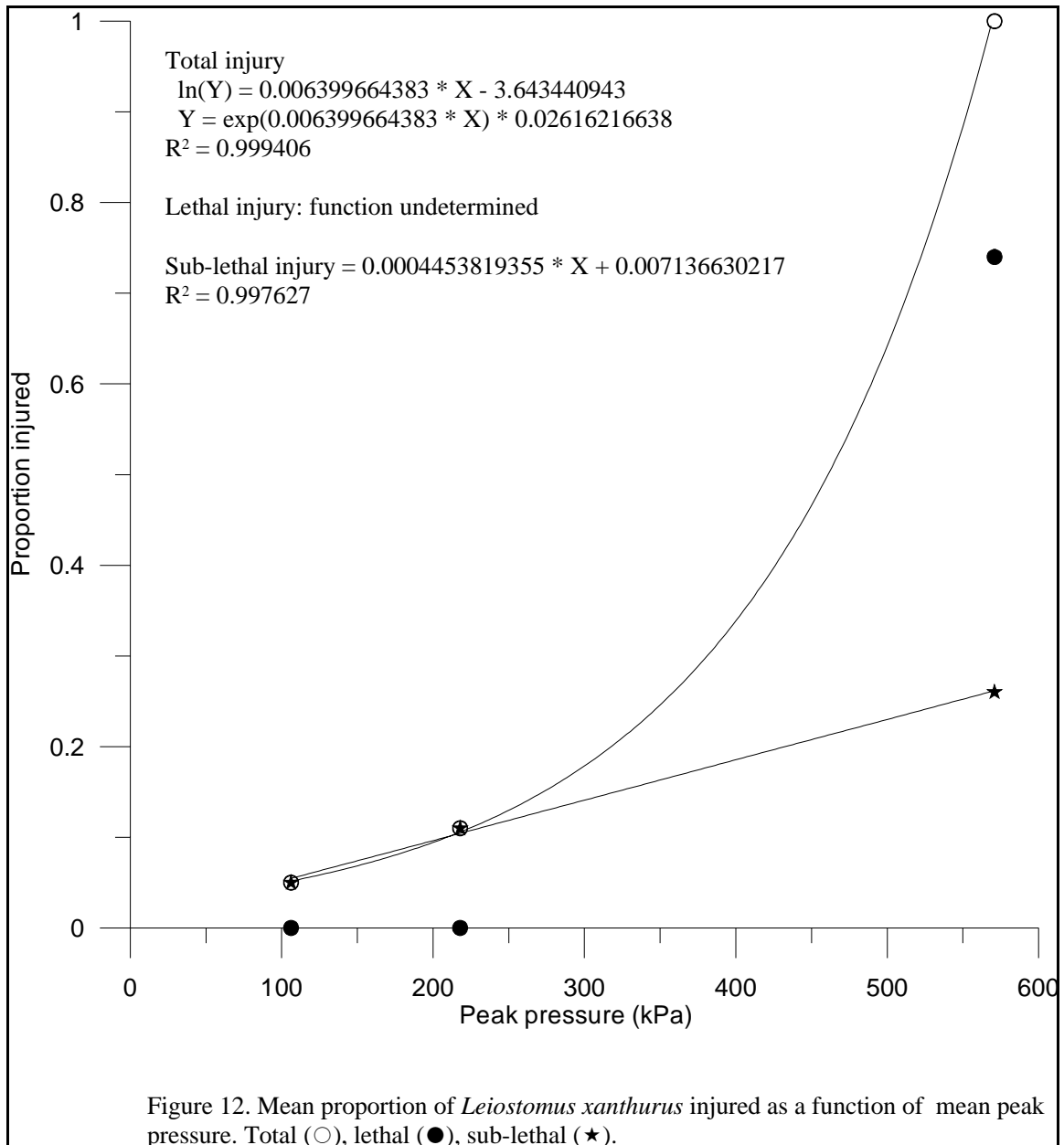


Figure 8. Injuries observed in (A) pinfish, *Lagodon rhomboides*, and (B) spot, *Leiostomus xanthurus*, exposed to underwater shock waves. CH - coelomic hemorrhage, HEM - hematuria of the kidney, SBH - swimbladder hemorrhage, LH - liver hemorrhage, LN - liver necrosis, RP - ruptured pancreas. Alimentary canal necrosis, a condition unrelated to shockwave exposure is not included. Not shown are two control pinfish with coelomic hemorrhage. None of the other injuries were observed in the controls.









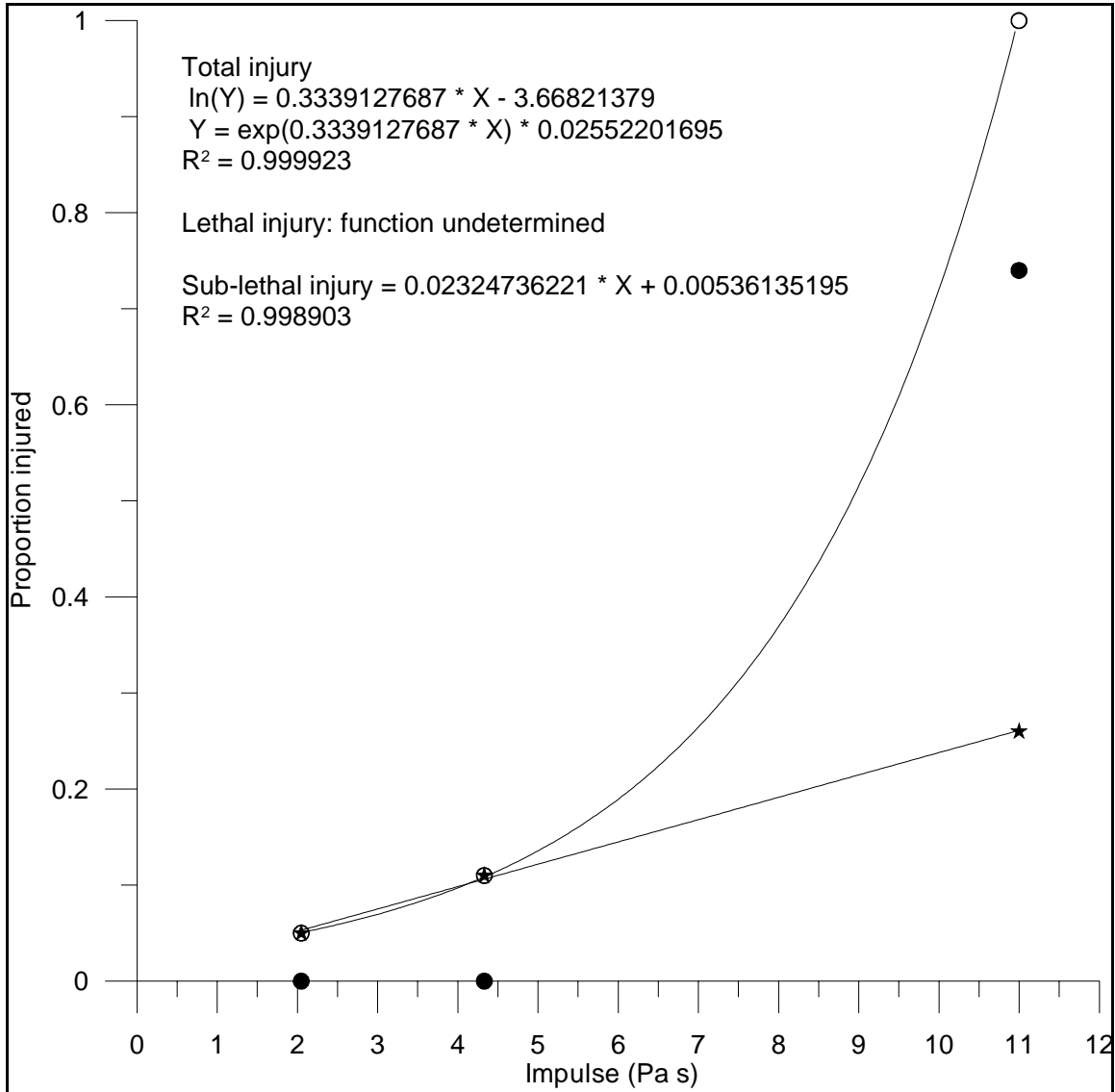


Figure 13. Mean proportion of *Leiosomus xanthurus* injured as a function of impulse. Total (○), lethal (●), sub-lethal (★).

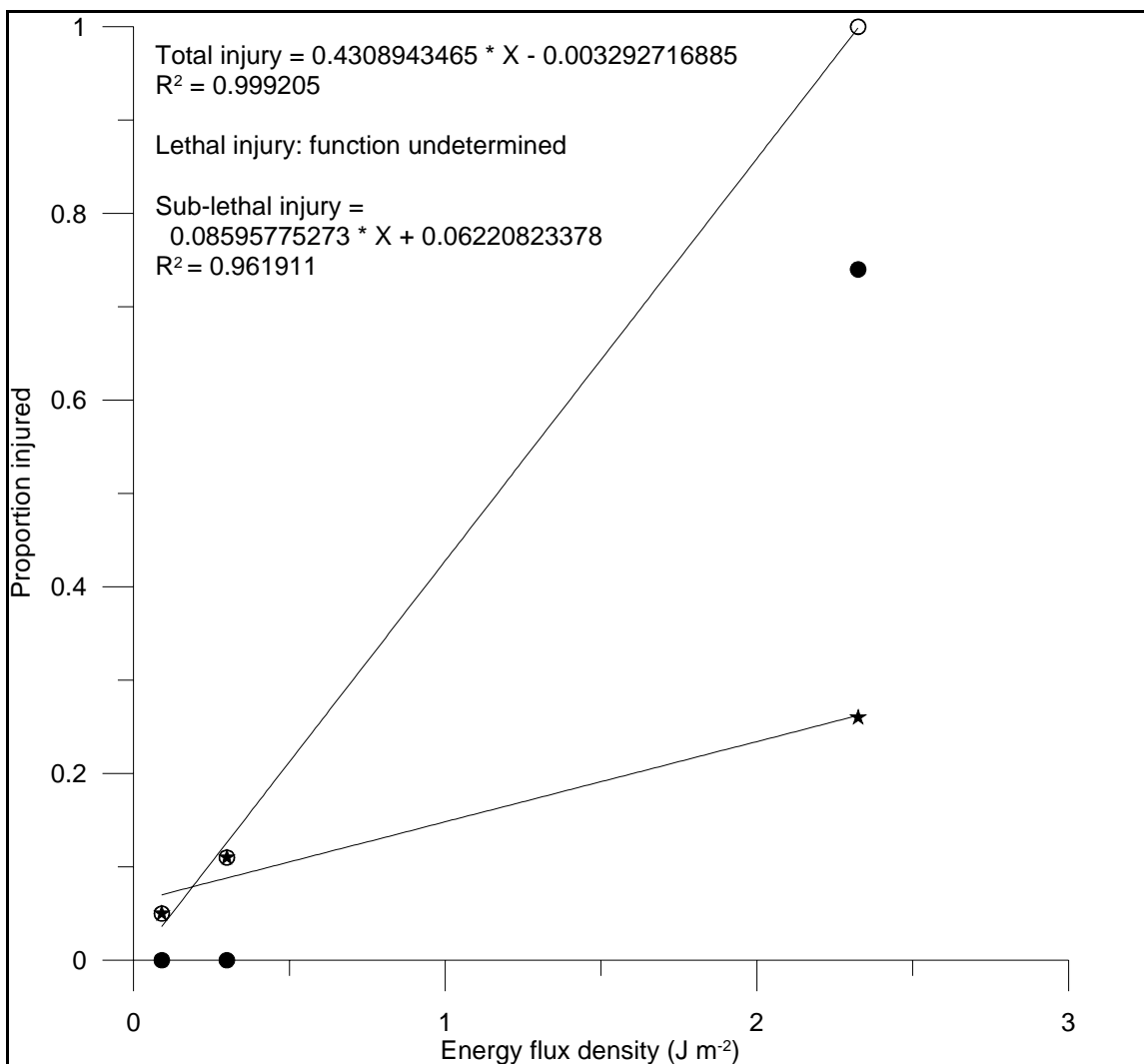
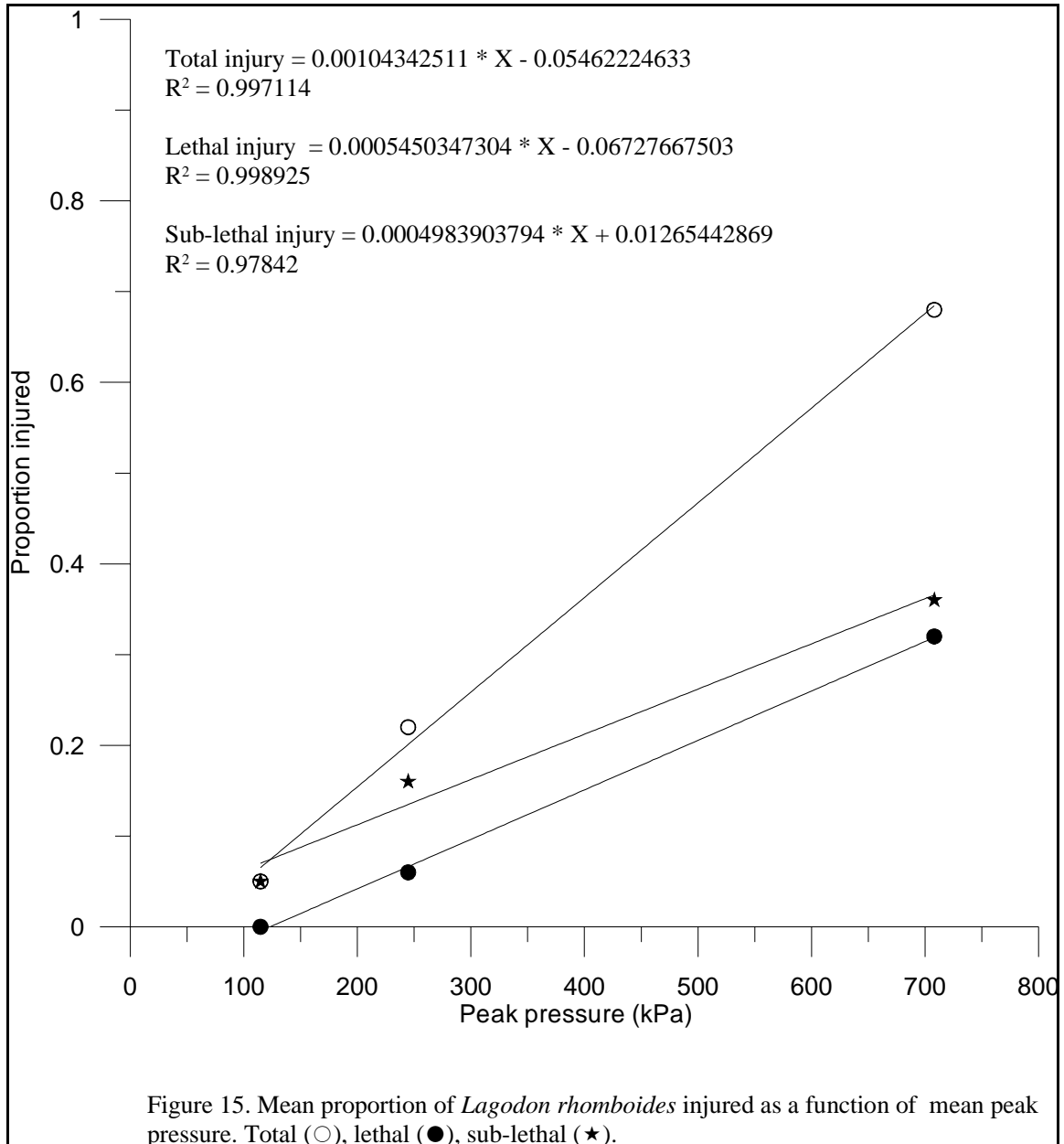
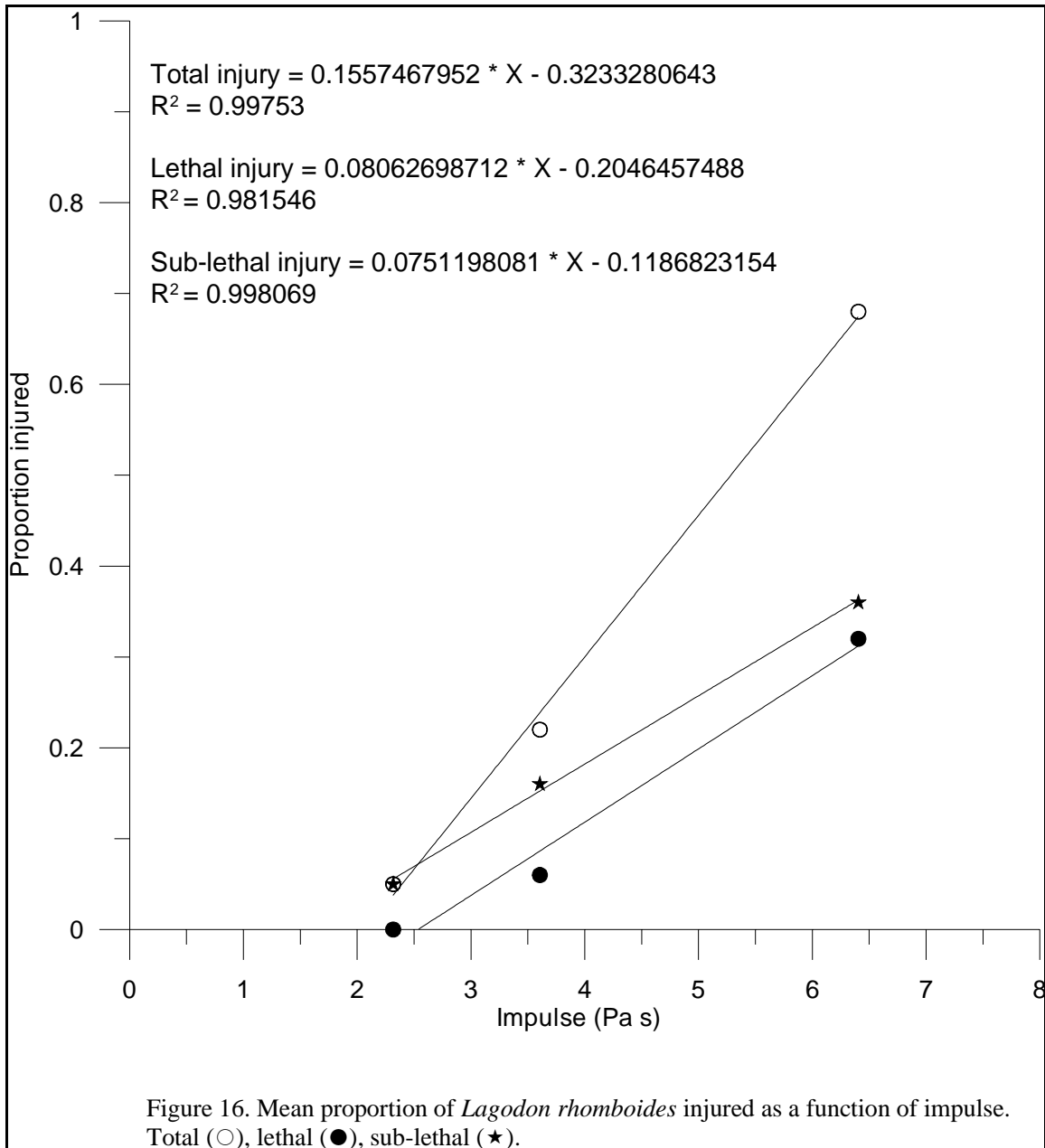
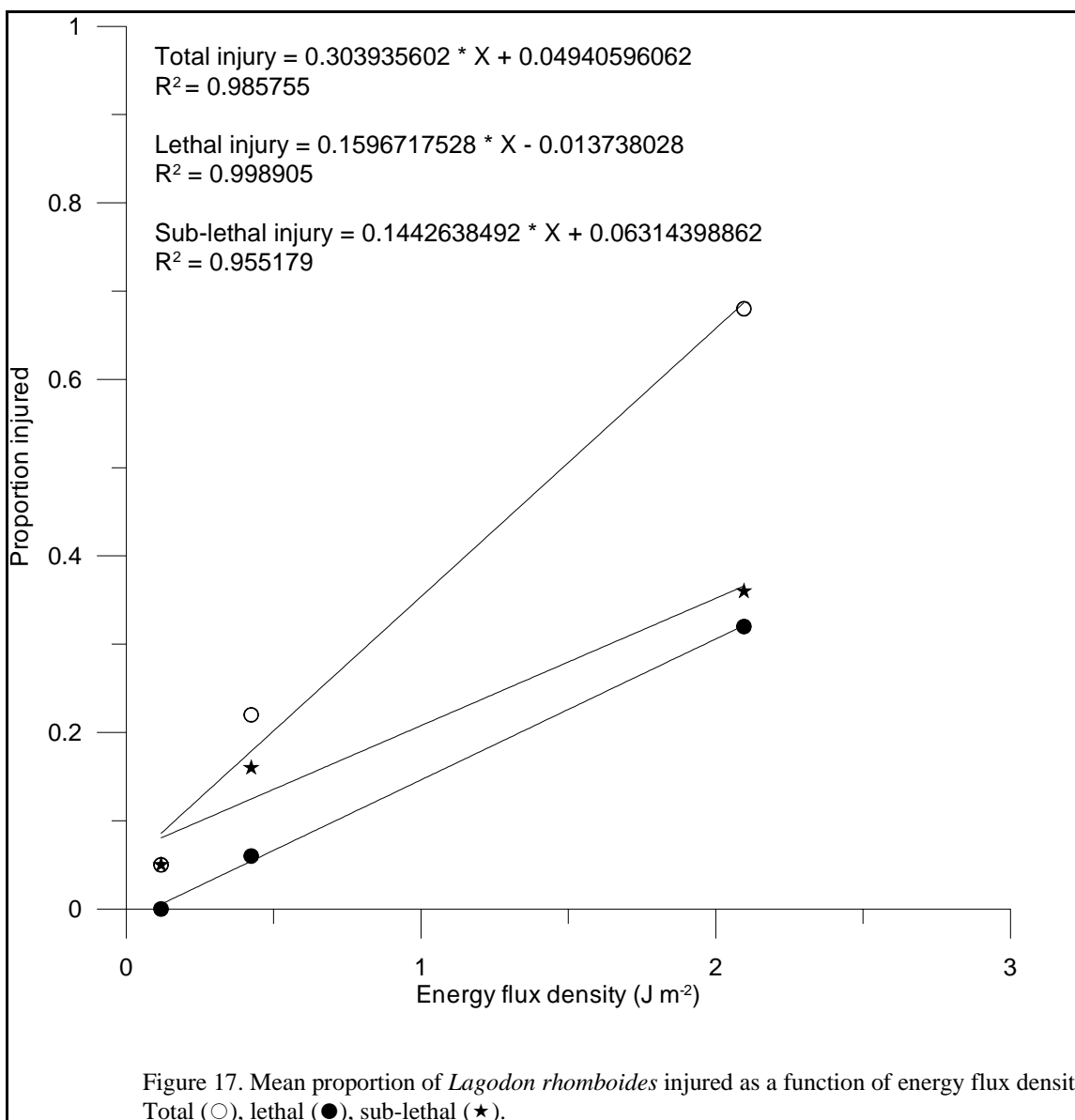


Figure 14. Mean proportion of *Leiosomus xanthurus* injured as a function of energy flux density
 Total (○), lethal (●), sub-lethal (★).







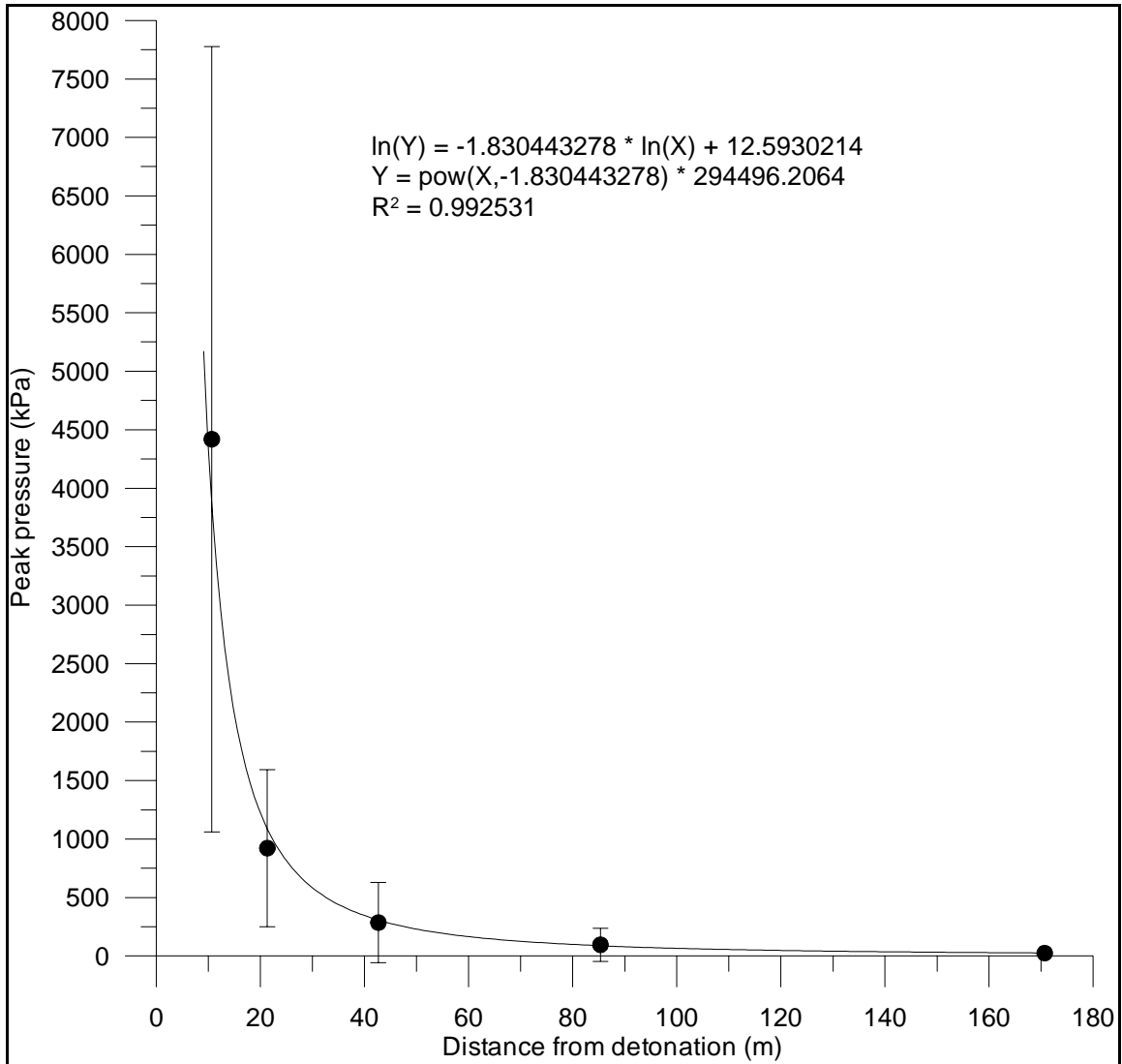
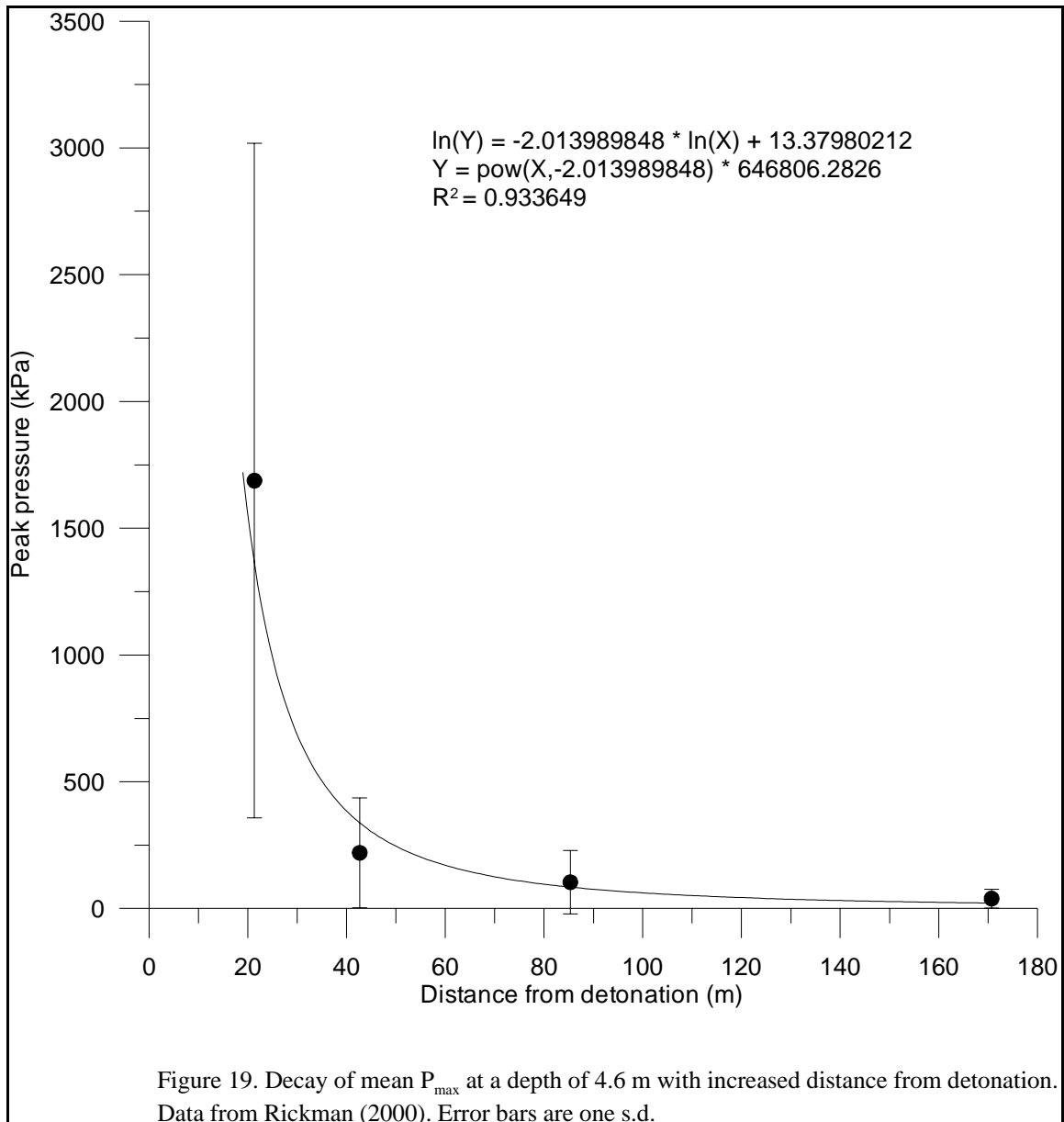
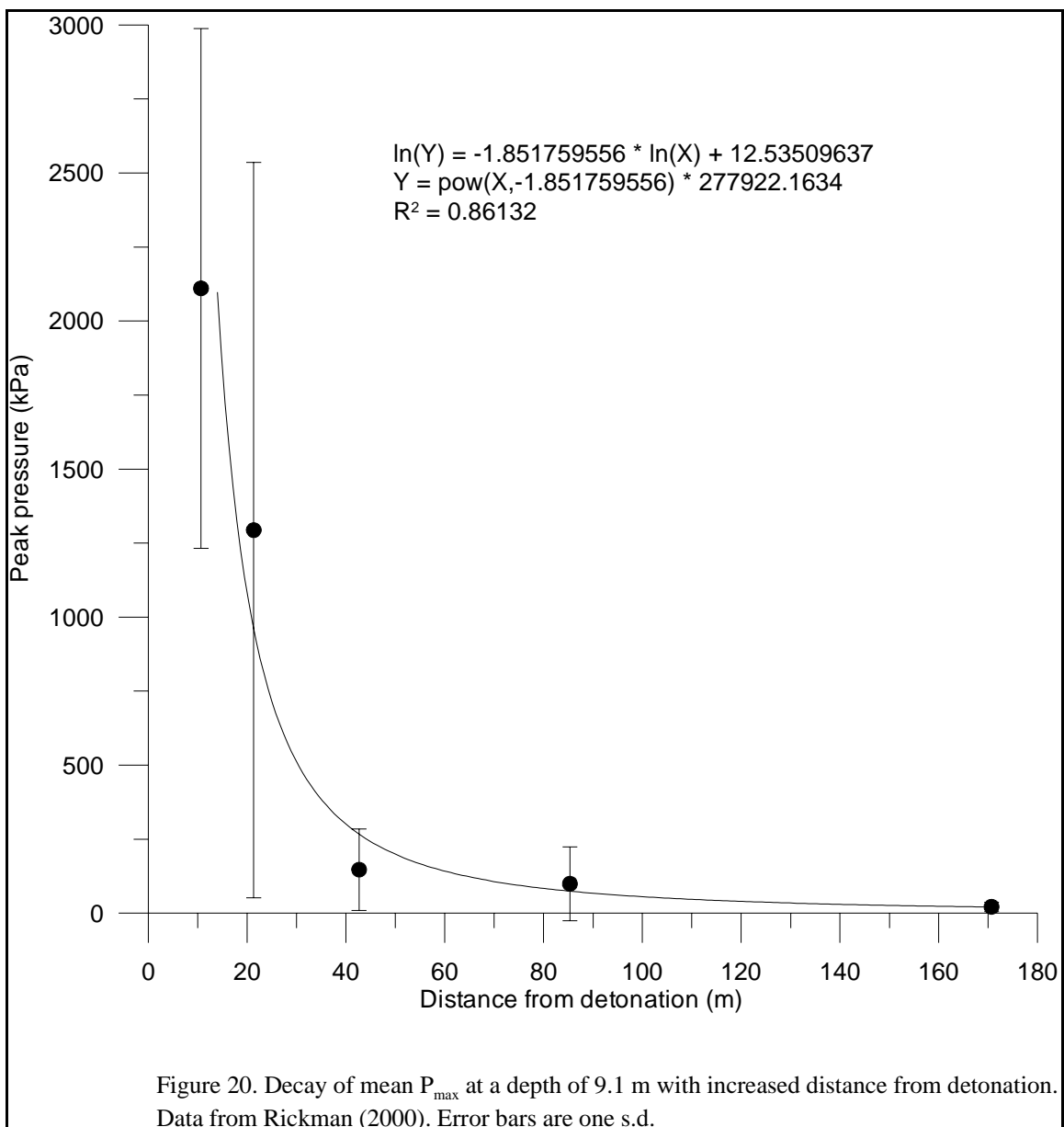


Figure 18. Decay of mean P_{\max} at a depth of 0.9 m with increased distance from detonation. Data from Rickman (2000). Error bars are one s.d.





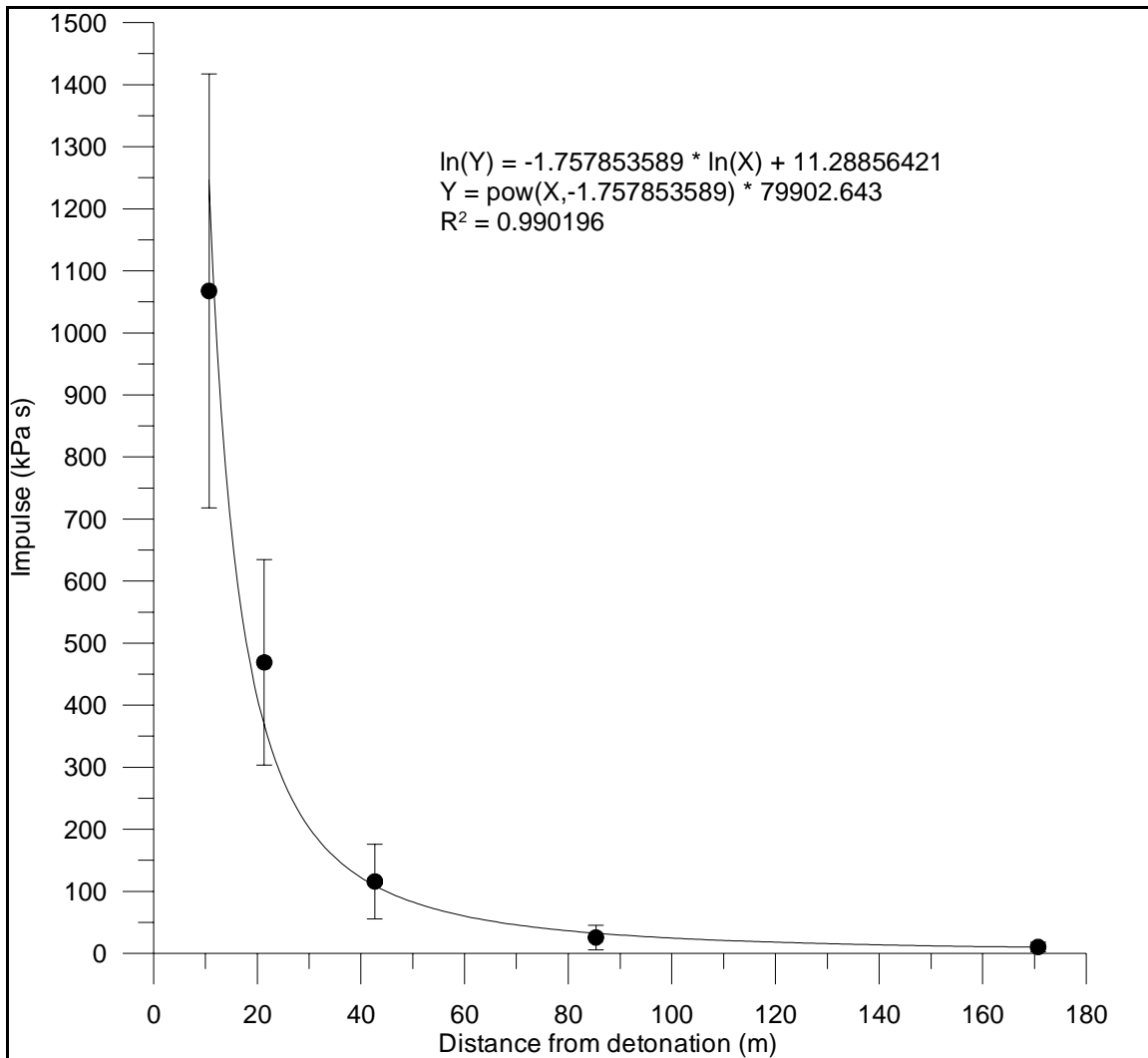


Figure 21. Decay of mean impulse at a depth of 0.9 m with increased distance from detonation. Data from Rickman (2000). Error bars are one s.d.

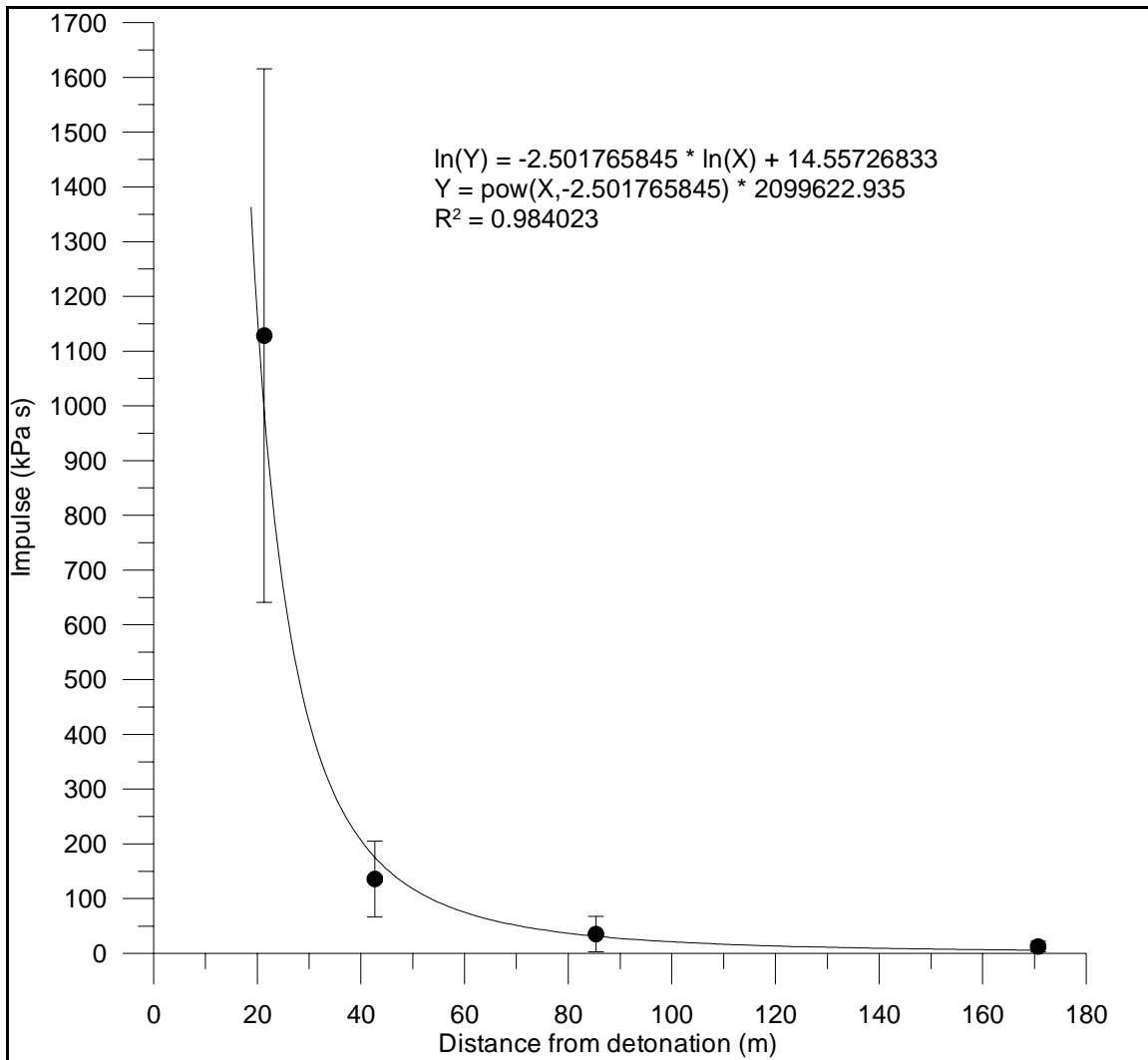


Figure 22. Decay of mean impulse at a depth of 4.6 m with increased distance from detonation. Data from Rickman (2000). Error bars are one s.d.

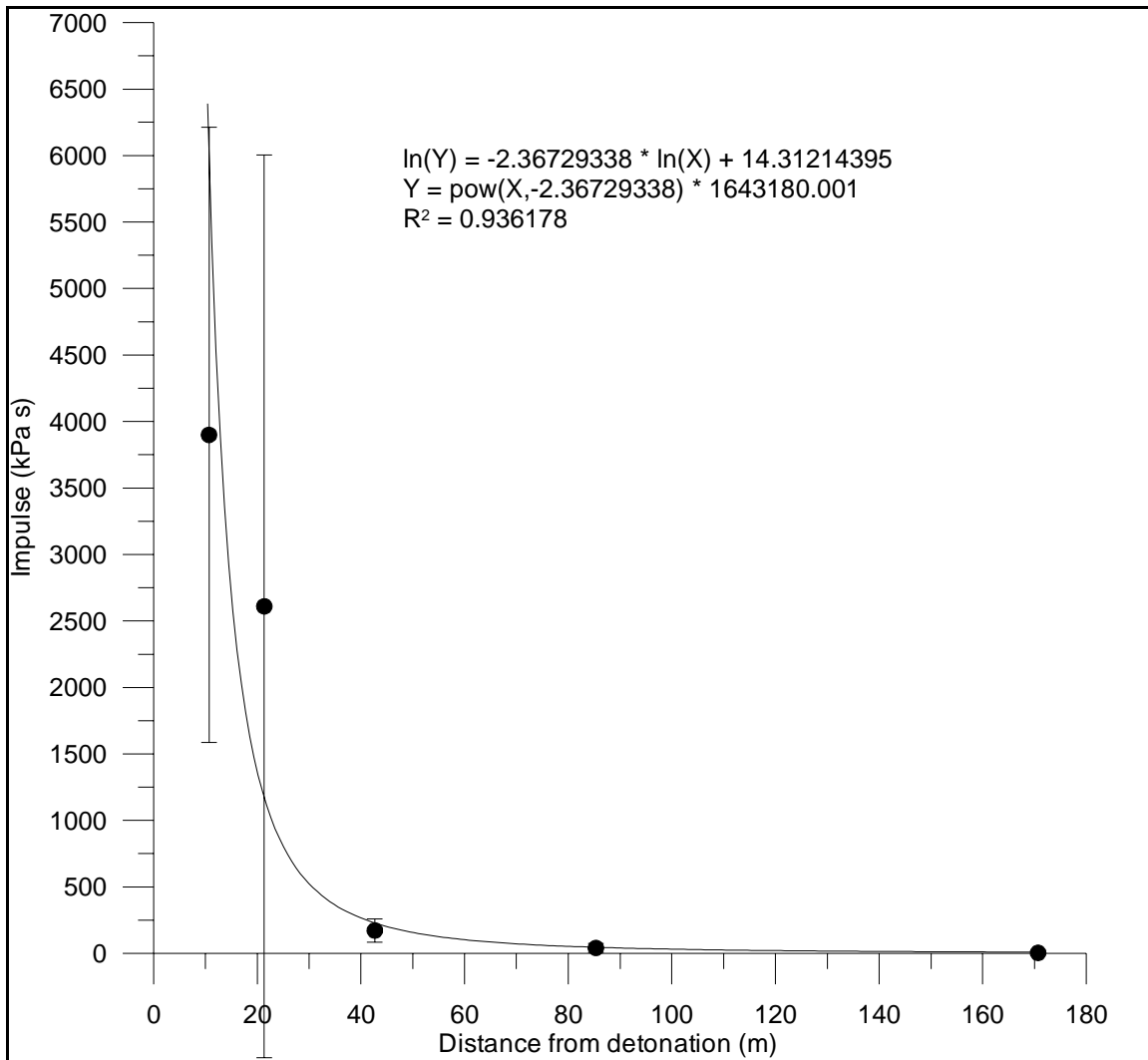
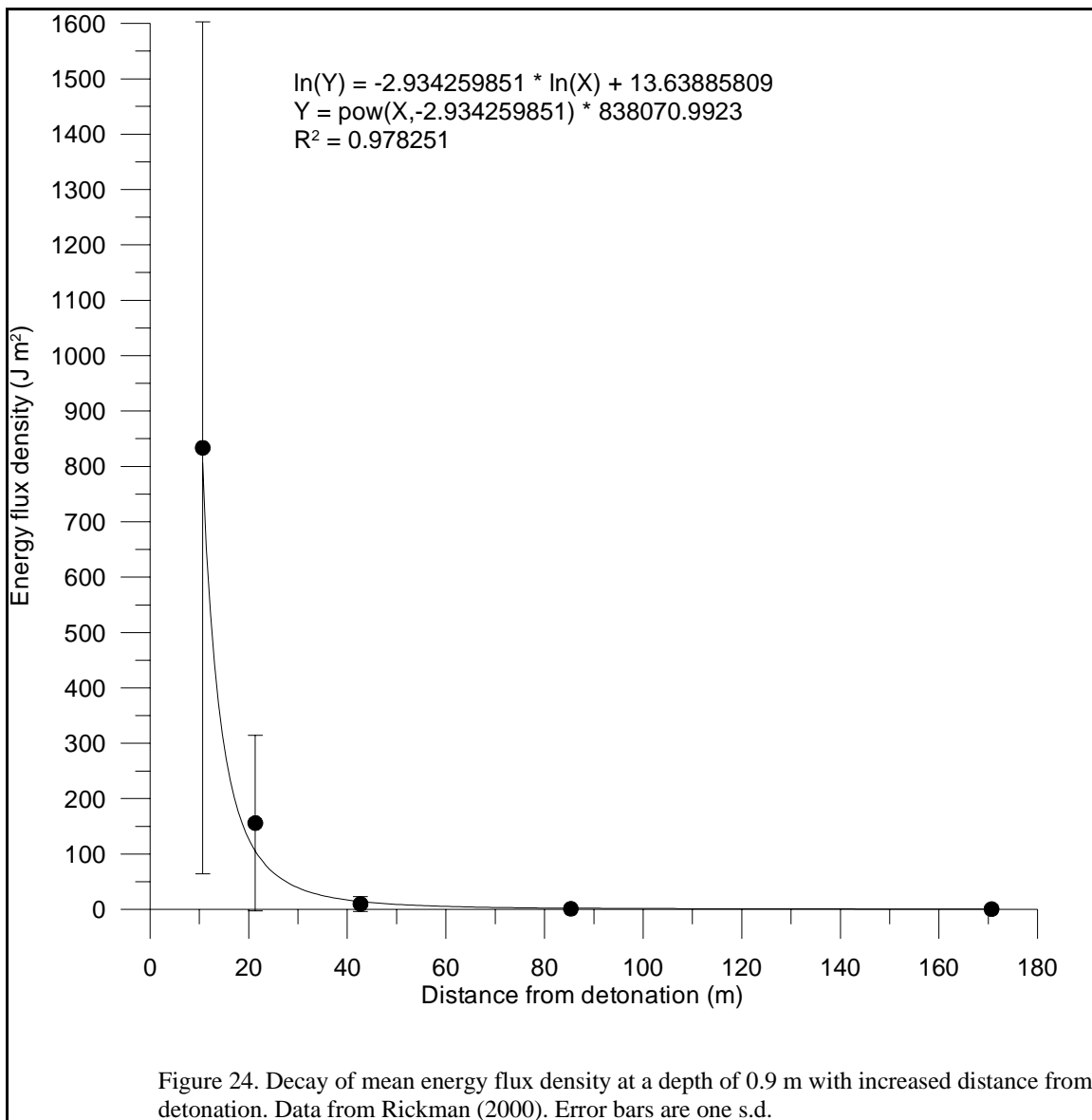
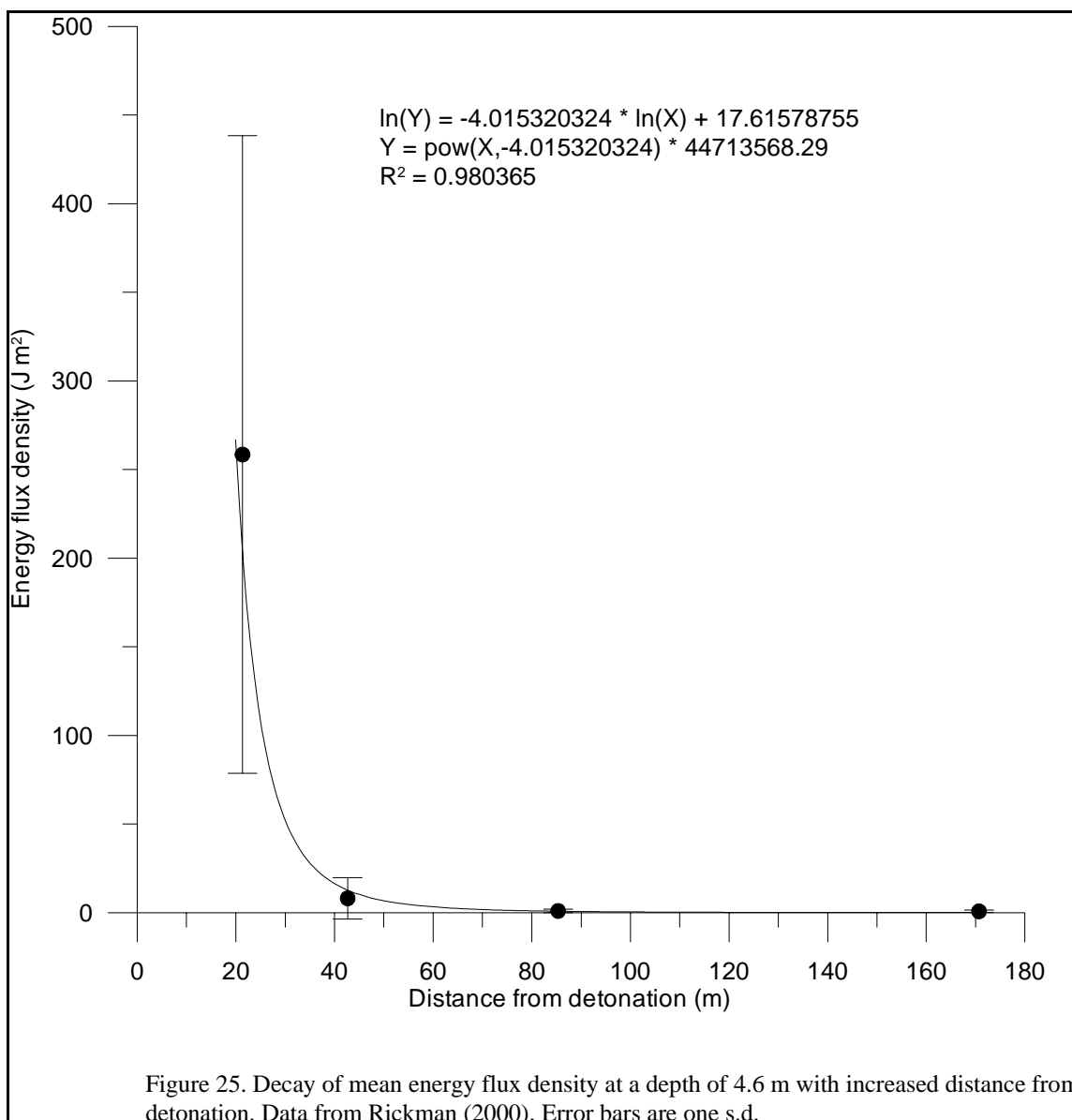
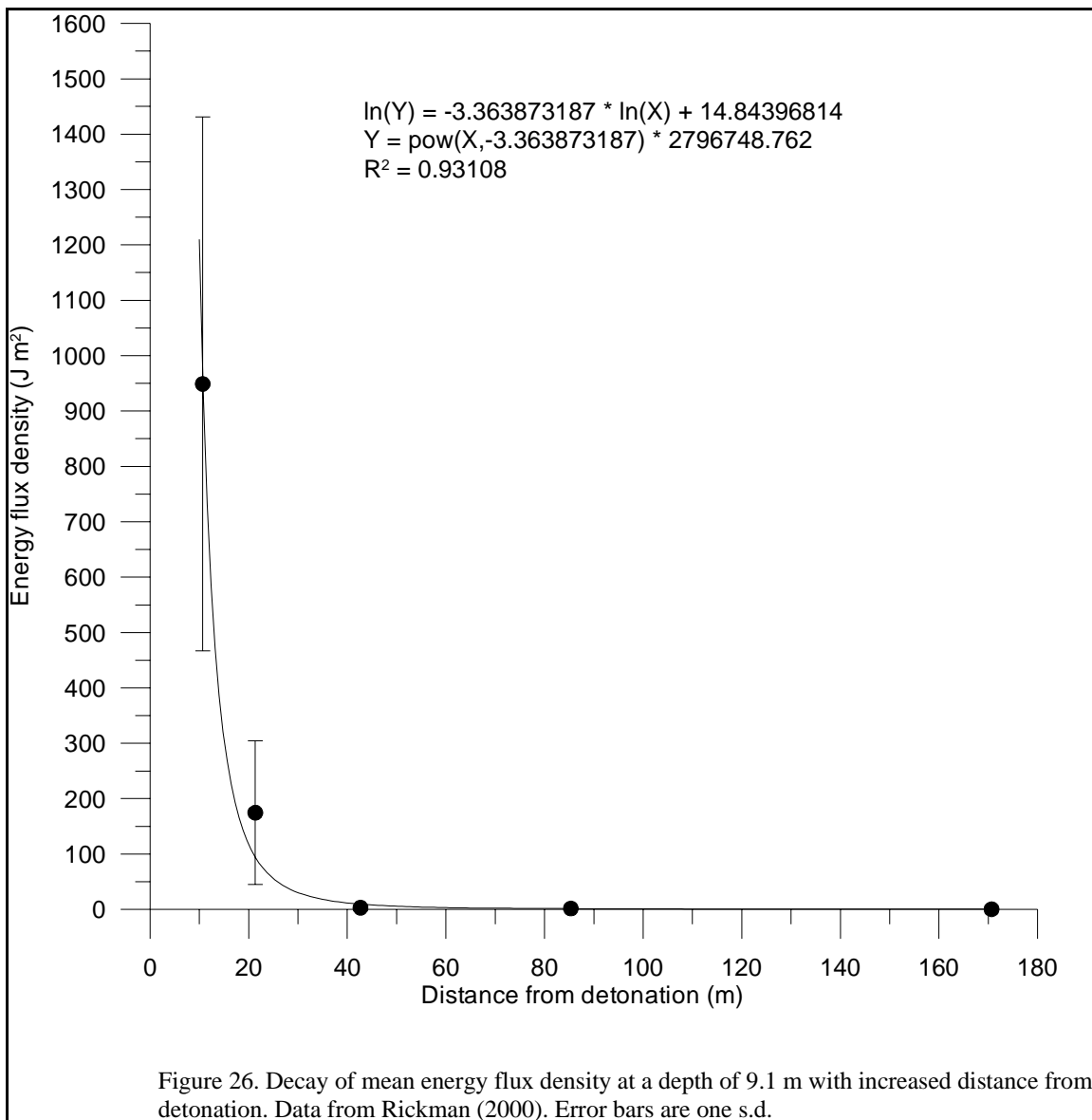


Figure 23. Decay of mean impulse at a depth of 9.1 m with increased distance from detonation. Data from Rickman (2000). Error bars are one s.d.







Investigation of impacts of underwater explosions on larval and early juvenile fishes

Part 2

Trauma to late-stage larval and early-juvenile pinfish, *Lagodon rhomboides*, and spot,

Leiostomus xanthurus, inflicted by sub-marine detonations

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Mention of trade names in this report does not confer endorsement by NOAA.

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Abstract

Juvenile pinfish, *Lagodon rhomboides*, and spot, *Leiostomus xanthurus*, exposed to pressure waves emanating from experimental underwater detonations exhibited both sub-lethal and probable antemortem trauma. Hyperemia within the swimbladder and liver, hematuria, coagulative liver necrosis, and rupture of the pancreas were the most recurrent and significant traumas evident from histopathological examination, and were the only ones attributed to exposure to pressure waves. These traumas were likely caused by rapid compression and expansion of the swimbladder as the impulse passed. Of these traumas, hyperemia within visceral organs and hematuria are likely sub-lethal. Rupture of the pancreas and coagulative liver necrosis are typically irreversible and hence probably antemortem.

Introduction

Previous assessments of the effects of sub-marine detonations on fishes have been limited by the size of fish assessed and to the morphological level of assessment. Fishes exposed to pressure waves that emanate from detonations, either experimental or through *in situ* engineering or military projects, have been larger than 54 mm in length (Wiley et al. 1981) or 0.02 g body weight (Yelverton et al., 1975). Effects of exposure have been assessed typically by mortality estimates and trauma at the gross anatomical level. Extrapolation of trauma to fishes smaller than 54 mm is tenuous, because larval and small juvenile fishes appear to be more sensitive than are adults to insult of any kind, including increases in pressure (Bishai 1961) and exposure to pressure waves (Fitch and Young 1948). Moreover, the swimbladder, an internal gas-filled vessel that reacts instantaneously to changes in ambient pressure, is thinly lined and more distensible in larval and juvenile fishes than it is in adults (Govoni and Hoss 2001). No histopathological assessment of trauma potentially caused by pressure waves is available for any fish of any size, while assessment at this level is the only accurate method of assessment for fish larvae and small juveniles.

Trauma currently recognized as resulting from sub-marine detonations are typically contusions of external anatomy or visceral organs, rupture of visceral organs, and hemorrhage. Hubbs and Rehnitz (1952) and Linton et al. (1985) diagnosed rupture specifically of the peritoneum, liver, kidneys, spleen, gallbladder, alimentary canal, and swimbladder. Hemorrhage has been observed within the coelomic cavity or within or about the liver, kidneys, spleen, and swimbladder (Hubbs and Rehnitz 1952; Linton et al. 1985).

The principal cause of trauma evidenced in the external anatomy is the impact of the pressure (P) wave (shock front), measured as the energy flux density ($EFD = (\rho c)^{-1} \int P^2 dt$; where P=pressure ρ =seawater density, c=velocity of sound in seawater, and t, time); the cause of trauma to the viscera is rapid compression and expansion, of the swimbladder as the pressure wave, or impulse ($I = \int P dt$), passes (Wiley et al. 1981). Maximum pressure (P_{max}), above ambient, occurs initially when the shock front passes; minimum pressure (P_{min}) occurs at rarefaction, after the passage of the shock front, and typically results from reflection from the air - water interface (Yelverton et al., 1975).

Controlled, experimental, sub-marine detonations that produced pressure waves commensurate with these produced by current blasting and dredging projects (Settle et al., 2002) exposed transforming larval or young juvenile pinfish, *Lagodon rhomboides*, and spot, *Leiostomus xanthurus*, to pressure waves and provided histopathological assessment of injury. Here we report sub-lethal and antemortem traumas that result from these experimental detonations.

Methods

Methods for the experimental detonations followed Wiley et al. (1981), as modified by Settle et al. (2002). Before exposure to pressure waves from detonations, pinfish, 13.8 to 21.3 mm standard length (SL), and spot, 15.1 to 25.3 mm SL, were collected in a tidal passage off the northwest end of Pivers Island, Beaufort, North Carolina; held in the laboratory in flowing seawater at ambient temperature and salinity (~14° C and 28-30 psu) with a 12:12 h light-dark cycle; and fed pelletized fish feed (COREY HI-PRO Starter and Fry Feeds (fish meal, fish oil,

wheat, krill, salt, vitamins, minerals, pigments, methionine)). The constitution of visceral organs indicate that spot begin transformation from larvae to juveniles at about 7 mm and complete transformation at 14 mm SL (Govoni 1980); we assumed the same for pinfish. Therefore, the subjects of these experiments were transforming larval and young juvenile fish. The evening before experimental detonations, 500 fish were removed from holding tanks, introduced into 24, 2 mil polyethylene bags in 5 L of seawater under constant aeration, and held overnight. The following morning, dead or moribund fish were removed from the bags and the bags sealed. Bags with healthy fish were moved to the same channel from which fish were collected for exposure to pressure waves emanating from experimental detonations.

The form of the pressure wave generated by experimental detonations compared well with those reported by Yelverton et al.(1975) with characteristic P_{\max} , I , and EFD (see Lynch and Revy, 2002). From Yelverton et al. (1975), an average I of $4.7 \text{ Pa} \cdot \text{s}$ is the threshold for measurable impact on small fish, whereas average I generated by detonations herein ranged from ~ 2.0 to $8.5 \text{ Pa} \cdot \text{s}$ (Table 1).

Bags with fish were submerged to 2.0 m depth, at three distances from the detonation; 3.6, 7.5, and 17.0 m. Fish were not allowed to equilibrate swimbladder volume to the pressure encountered at 2.0 m, because of equilibration periods are in the order of hours; for spot $>2\text{h}$ (Govoni and Hoss 2001). Triplicate charges were detonated with one bag of each fish species submerged at each distance and for each detonation. Control bags, one for each species, were submerged subsequently for 7 min without detonation.

Following detonations, bags were retrieved, and the number of dead fish and the behavioral condition of live fish recorded (Settle et al., 2002). Bags were moved to the

laboratory, opened, and the number of dead fish and the behavioral condition of live fish again recorded. Fish showing no signs of gross anatomical trauma or aberrant behavior were held in aerated seawater at 14° C and re-examined at 4 and 24 h thereafter. Dead and moribund fish examined immediately after exposure and at 4 and 24 h junctures were examined under a stereo-microscope, measured (mm SL), and preserved in histological grade neutral buffered (phosphate) formalin. Samples of living fish exposed and not exposed (controls) at 24 h were examined, measured, and preserved. Moribund and living fish were anesthetized with MS-222 (tricaine methanesulfonate: FINQUEL—ARGENT Chemical Laboratories, Redmond Washington) before examination and fixation.

The anesthetic effect of MS-222 is brought about by depression of the medulla of the brain, which in turn depresses respiration and causes blood hypoxia (Smith et al. 1999). Aside from some changes in erythrocyte morphology, there is no known histopathology associated with anesthesia using MS-222 (Smit et al. 1979). To assess this further, 2 pinfish and 2 spot, neither exposed to pressure waves nor treated as controls, were anesthetized with the same concentration of MS-222 and fixed for histopathology. None displayed any of the traumas reported herein.

For histopathology, preserved fish were divided into two groups; <20 mm and >20 mm. Smaller fish were decalcified in 10% formic acid for 24 h; larger fish for 2 d. Fish were prepared by standard procedures for paraffin embedding and sectioning. Ten to 18, 5 μ m, parasagittal sections (including the medial) were cut from each fish with five to six sections each at 50 μ m intervals. Sections were stained with Mayer's-Harris Hematoxylin and counter stained with Eosin y-phloxine.

Results

One pinfish and six spot were partially eviscerated when examined in the laboratory after experimental detonations. In addition, two exposed pinfish and 14 exposed spot exhibited autolysis of viscera; 3 control spot did as well. All fish that exhibited autolysis were dead before preservation and exhibited poor staining reactions of histological sections typical of autolysis. These partially eviscerated and autolytic fish were excluded from histopathological assessment.

No other external lesions were evident in exposed fish, but internal hemorrhaging was evident from gross examination in five pinfish exposed 3.6 m from detonations and one exposed at 17.0 m. Four spot showed internal hemorrhaging at 3.6 m. No fish from controls showed internal hemorrhaging from gross examination.

Both exposed and control pinfish had aggregations of erythrocytes in the caudad dorsal coelom indicating hemorrhage, but these aggregations could not be related to rupture of specific arteries or veins. While this hemorrhage appeared more extensive in exposed fish than in control fish, the incidence of coelomic hemorrhage (Table 2) was independent of exposure at any distance from the detonation (frequency analysis with replicates pooled, Fisher's Exact Test, $P \leq 1.000$ for distance from detonation and $P \leq 0.6081$ for exposure). Leucocytes were more prevalent in regions exhibiting coelomic hemorrhage among control pinfish when compared with fish exposed to pressure waves (Figs. 1 A and B), but the ratio of erythrocytes to leukocytes in exposed control pinfish were not significantly different ($\chi^2 = 2.4050$; $P \leq 0.1209$). Although visceral hemorrhage is a trauma often attributed to exposure of large juvenile and adult fishes to pressure waves that emanate from sub-marine detonations (Hubbs and Rechnitzer 1952; Linton et al. 1985), aggregations of erythrocytes observed here may have resulted from compression and

subsequent expansion of the swimbladder when pinfish were lowered and raised to and from 2 m depth (Table 3). While swimbladder volumes (Table 3) are for spot, the shape and volume of the swimbladder of pinfish are similar.

Exposed pinfish and spot had hyperemia within the swimbladder serosa (Fig. 2A), the mucosa of the gas gland, and the rete mirabile (Figs. 2B), and within the liver (Fig. 3). Exposed fishes only (Table 2) had aggregations of erythrocytes in interstices of these tissues. Hyperemia within the liver was evident in regions proximal to the swimbladder. Contusion of the liver in the region proximal to the swimbladder was also evident in one exposed spot.

The kidney tubules of exposed pinfish and spot (Table 2; Fig.4) exhibited hematuria with erythrocytes in the lumen of the proximal kidney tubules. No control fish evidenced hematuria.

Apparent liquifactive necrosis was evident in the mucosa of the dorsal region of the anterior intestine of exposed and control pinfish and spot (Table 2). No pinfish and 8% of spot that evidenced this intestinal necrosis were dead before fixation. Frequencies of necrosis of the anterior intestine were dependent upon exposure to pressure waves, but with no significant differences among distances from detonation. The exact probability of observing a frequency distribution this extreme was 0.0254 for pinfish and 0.0182 for spot.

Pinfish and spot exhibited two types of liver necrosis: apparent liquifactive and coagulative (Table 2). Only control spot exhibited liquifactive necrosis of the liver. Liquifactive necrotic liver tissue was not associated with hyperemia and was less eosinophilic than tissue that had coagulative necrosis. Coagulative liver necrosis was evident only in exposed pinfish and spot (Table 2) and occurred proximal to the swimbladder (Fig 5A). Coagulative necrotic infarcts were evident as areas of cellular disorganization where cell membranes were ill-defined and

always proximal to areas of hyperemia within the liver (Fig. 5B). Over-abundance of erythrocytes in one area creates ischemia in the adjacent area, the necrotic zone.

Of the viscera, only the pancreas ruptured in exposed pinfish and spot (Table 2). The mesentery surrounding the pancreas was disrupted and pancreatic zymogen granules were present in the interstices surrounding the organ. Rupture was always evident in regions where the organ was proximal to the swimbladder. No control fish had a ruptured pancreas (Fig. 7).

Discussion

Most of the traumas revealed herein would not have been identified or adequately described by gross anatomical examination; coelomic hemorrhage is the single exception. The small size of larval and juvenile fishes, along with the attributes of the traumas observed, necessitates histopathology.

Hyperemia within the swimbladder and liver, hematuria, coagulative liver necrosis, and rupture of the pancreas were the most recurrent and pathologically significant traumas evident, and the only ones attributed to exposure to sub-marine detonations. These recurrent, pathological traumas were likely caused by the rapid compression and subsequent expansion of the swimbladder as the pressure wave passed. This movement disrupts the serosa and surrounding mesentery of the pancreas and the endothelium of arterioles and venules, and probably causes leaking of erythrocytes into the lumen of kidney tubules (hematuria). Rapid compression of the liver can cause disruption of cell membranes, which along with localized ischemia, causes coagulative liver necrosis.

Other observed traumas, hemorrhage and liquifactive necrosis, were not attributed to exposure to pressure waves. Hemorrhage was evident in exposed as well as control fishes and may have resulted from compression and expansion of the swimbladder as fishes were lowered to depth and retrieved. Of the two types of necrosis, only coagulative necrosis can be attributed to exposure to pressure waves. The apparent liquifactive necrosis of the mucosa of the alimentary canal and of the liver, observed in exposed and control pinfish and spot, were probably related to high food intake in that these lesions resembled those reported by Mobin et al. (2000; 2001). Liquifactive necrosis of the liver may be caused by permeation of digestive enzymes, released from the damaged alimentary canal, into proximal liver tissue (Mobin 2001). Herein, juvenile pinfish and spot were held in the laboratory before exposure to detonations for up to one week and were fed pelletized fish feed *ad libitum*. Liquifactive necrosis can not be attributed to exposure to pressure waves because the amount of actual food intake was not known. Coagulative liver necrosis, evident only in exposed fish, differed from liquifactive necrosis in that lesions had intra-tissue hyperemia and were always proximal to the swimbladder.

Wiley et al (1981) devised a dynamic model of injury to fishes caused by sub-marine detonations that embodies swimbladder compression and expansion. In this model, injury results from oscillations of the swimbladder volume that lag P_{max} of the pressure wave. Minimum volume is reached soon after P_{max} ; maximum volume after rarefaction. This model assumes discrete, rather than a continuous, decrease in pressure after the passage of the P_{max} , while oscillations in swimbladder volume result from the step function. Impact injury to the external anatomy might result from the passage of a singular wave form. Rapid compression and expansion of the swimbladder, whether static or oscillatory, can cause trauma to internal organs,

if the expansion and contraction is voluminous. Our data does not depict oscillation of swimbladder volume, but trauma to visceral organs owing to disruption of viscera was clearly evident.

Pressure change experienced by transforming larval and young juvenile pinfish and spot were within the range reported as the threshold for injury to small fishes (Yelverton et al., 1975). These pressure changes were several orders of magnitude higher than those reported to have no injurious effect on fishes (Traxler et al. 1992).

With ambient temperatures and pressures recorded *in situ* during the experiments, P_{\max} and P_{\min} of the pressure waves encountered by these fish during exposure (Settle, et al., 2002), and the regression equation for swimbladder volume and SL of larval spot at atmospheric pressure given in Govoni and Hoss (2001), the swimbladders of juvenile spot exposed to pressure waves encountered here probably would have contracted by an order of magnitude, i.e. $\sim 10 \mu\text{l}$ and expanded by approximately $4 \mu\text{l}$ from resting swimbladder volumes of approximately $1.6 \mu\text{l}$ (Table 3). The disruption that resulted from this compression and expansion was sufficient to cause hyperemia within the swimbladder and liver, hematuria, coagulative liver necrosis, and rupture of the pancreas.

Stunned fish or fish with anomalous behavior, reported by Settle et al. (2002), may indicate trauma or even the likelihood of death, but the imminence of death requires post-experimental observation longer than 24 h. Moreover, the determination of the lethality of injury requires comprehensive assessment of the severity or extent of the injury, which can not be inferred from histopathological examination. Of traumas evident here hyperemia and hematuria, are likely sub-lethal; juvenile pinfish and spot may recover. Rupture of the pancreas and

coagulative liver necrosis is typically irreversible and possibly antemortem. Behavioral abnormalities that might owe to these sub-lethal and antemortem traumas could render these fish more susceptible to predation in nature, but this potential mortality was not apprehensible.

Species-specific differences in the susceptibility to injury from sub-marine detonations are evident for large juvenile and adult fishes (Hubbs and Rehnitz 1956; Wiley et al. 1981; Linton et al. 1985). Small juvenile spot are more susceptible to sub-lethal and antemortem trauma than are pinfish; 46% of spot exhibited sub-lethal and antemortem trauma versus 31% of pinfish.

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TABLE 1.—Average pressure maximum (P_{\max}) and minimum (P_{\min}), energy flux density (E), and impulse(I) experienced by juvenile pinfish, *Lagodon rhomboides*, and spot, *Leiostomus xanthurus*, exposed to sub-marine detonations.

Pressure wave	Distance from Blast		
	3.6 m	7.5 m	17.0 m
P_{\max} (kPa)	636.92	230.86	109.93
P_{\min} (kPa)	-92.07	-70.77	-60.46
E ($\text{J} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$)	2.21	0.36	0.10
I ($\text{Pa} \cdot \text{s}$)	8.67	3.95	2.18

TABLE 2.—Observed trauma to juvenile pinfish, *Lagodon rhomboides*, and spot, *Leiostomus xanthurus*.

Trauma	Distance from Blast											
	3.6 m				7.5 m				17.0 m			
	Exposed		Control		Exposed		Control		Exposed		Control	
	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq	N	Freq
<i>Lagodon rhomboides</i>												
Coelomic hemorrhage ^a	23	2	6	0	19	1	6	1	19	1	6	1
Swimbladder hyperemia	23	5	6	0	19	0	6	0	19	0	6	0
Liver hyperemia	23	2	6	0	19	0	6	0	19	0	6	0
Hematuria	23	14	6	0	19	4	6	0	19	0	6	0
Alimentary canal necrosis ^a	23	4	6	4	19	5	6	2	19	2	6	2
Liquifactive liver necrosis ^a	23	0	6	0	19	0	6	0	19	0	6	0
Coagulative liver necrosis	23	2	6	0	19	0	6	0	19	0	6	0
Ruptured pancreas	23	2	6	0	19	0	6	0	19	0	6	0
<i>Leiostomus xanthurus</i>												
Coelomic hemorrhage ^a	27	0	7	0	18	0	9	0	18	0	6	0
Swimbladder hyperemia	27	8	7	0	18	0	9	0	18	0	6	0
Liver hyperemia	27	16	7	0	18	0	9	0	18	0	6	0
Hematuria	27	24	7	0	18	2	9	0	18	0	6	0
Alimentary canal necrosis ^a	27	11	7	5	18	6	9	6	18	6	6	3
Liquifactive liver necrosis ^a	27	0	7	0	18	0	9	2	18	0	6	1
Coagulative liver necrosis	27	11	7	0	18	0	9	0	18	0	6	0
Ruptured pancreas	27	4	7	0	18	0	9	0	18	0	6	0

^a Observed trauma not due to shockwave exposure.

TABLE 3.—Estimated swimbladder volume (L) of an 18.98 mm juvenile spot, *Leiostomus xanthurus*, before and during exposure to experimental sub-marine detonations.

Swimbladder volume	Distance from Blast		
	3.6 m	7.5 m	17.0 m
Estimated Resting Volume at Surface	1.58×10^{-6}	1.58×10^{-6}	1.58×10^{-6}
<i>In Situ</i> Estimated Resting Volume	1.31×10^{-6}	1.31×10^{-6}	1.31×10^{-6}
<i>In Situ</i> Volume at P_{\max}	2.09×10^{-7}	4.50×10^{-7}	6.87×10^{-7}
<i>In Situ</i> Volume at P_{\min}	5.42×10^{-6}	3.14×10^{-6}	2.62×10^{-6}

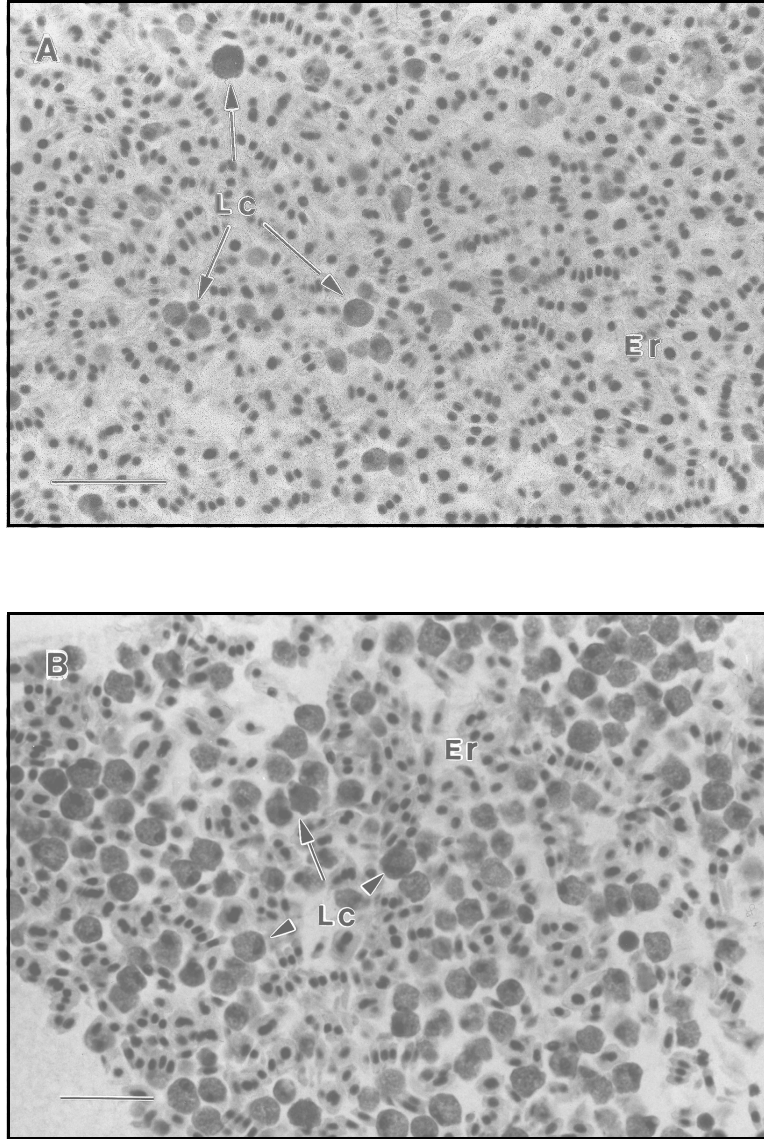


Figure 1.(A) Hemorrhage in the caudal dorsal coelom of a 16.7 mm SL pinfish, *Lagodon rhomboides*, exposed to a pressure wave from a sub-marine detonation (scale bar = 20 μm). (B) Hemorrhage in the caudal dorsal coelom of a 19.2 mm SL pinfish not exposed to a pressure wave from a sub-marine detonation (scale bar = 30 μm): Er, erythrocytes; Lc, leucocytes .

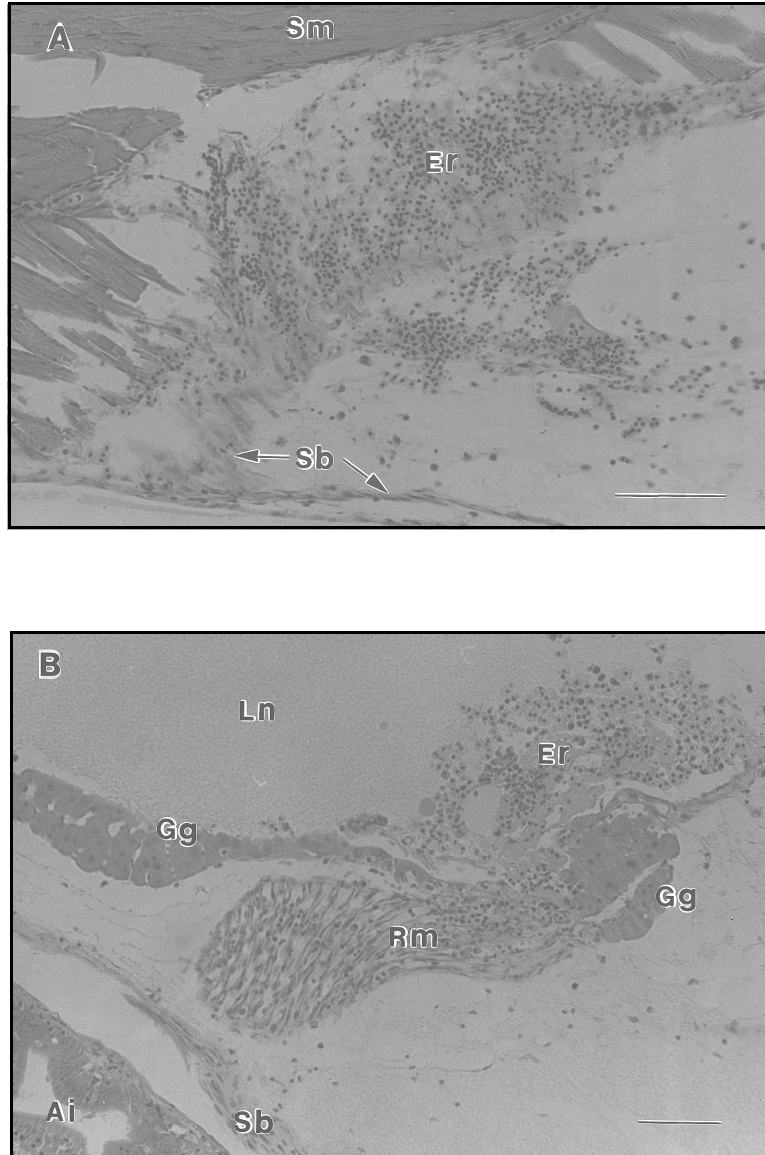


Figure 2.(A) Hyperemia of the posterior swimbladder serosa of a 21.2 mm SL spot, *Leiostomus xanthurus*, exposed to a pressure wave from a sub-marine detonation (scale bar= 75 μ m). (B) Hyperemia of the swimbladder gas gland tissue and rete mirabile of a 17.7 mm SL spot exposed to a pressure wave from a sub-marine detonation (scale bar = 45 μ m): Ai, anterior intestine; Er, erythrocytes; Gg, gas gland tissue; Ln, swimbladder lumen; Rm, rete mirabile; Sb, swimbladder serosa; Sm, striated axial muscle.

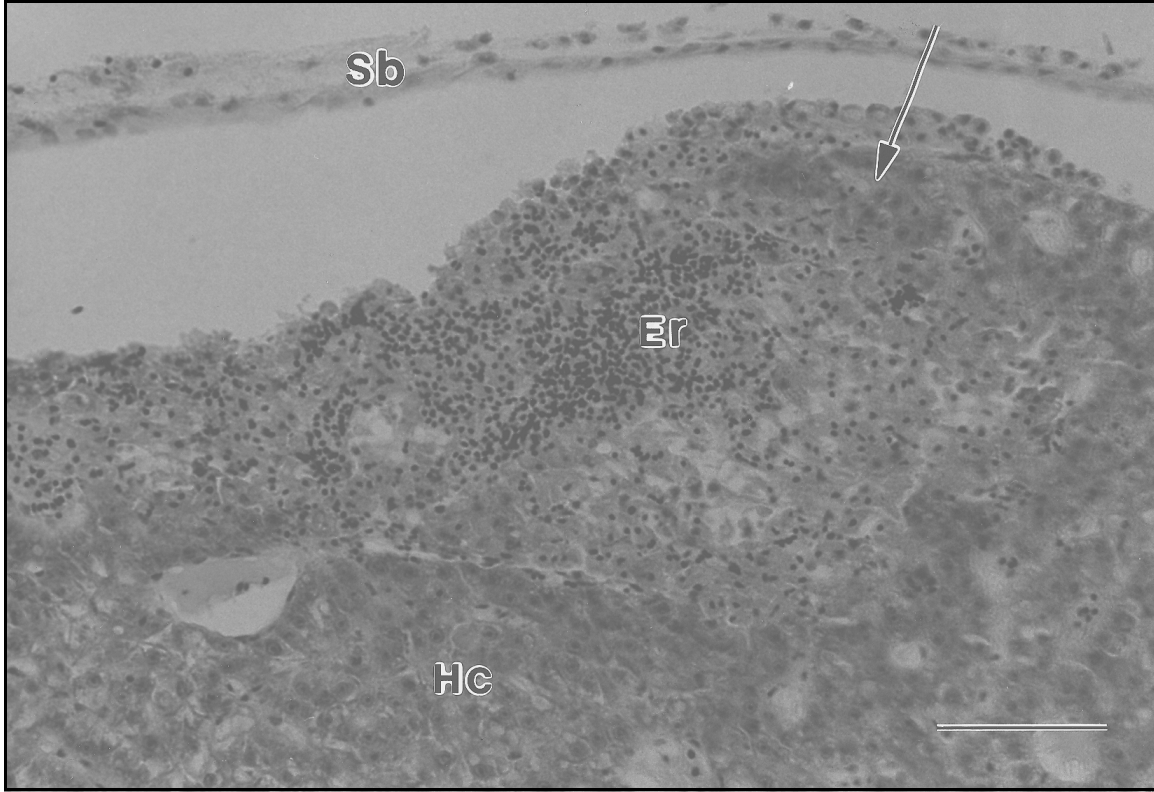


Figure 3. Hyperemia of the liver of a 21.2 mm SL spot, *Leiostomus xanthurus*, exposed to a pressure wave from a sub-marine detonation. The arrow indicates contusion (scale bar = 50 μ m): Er, erythrocytes; Hc, hepatocytes; Sb, swimbladder serosa.

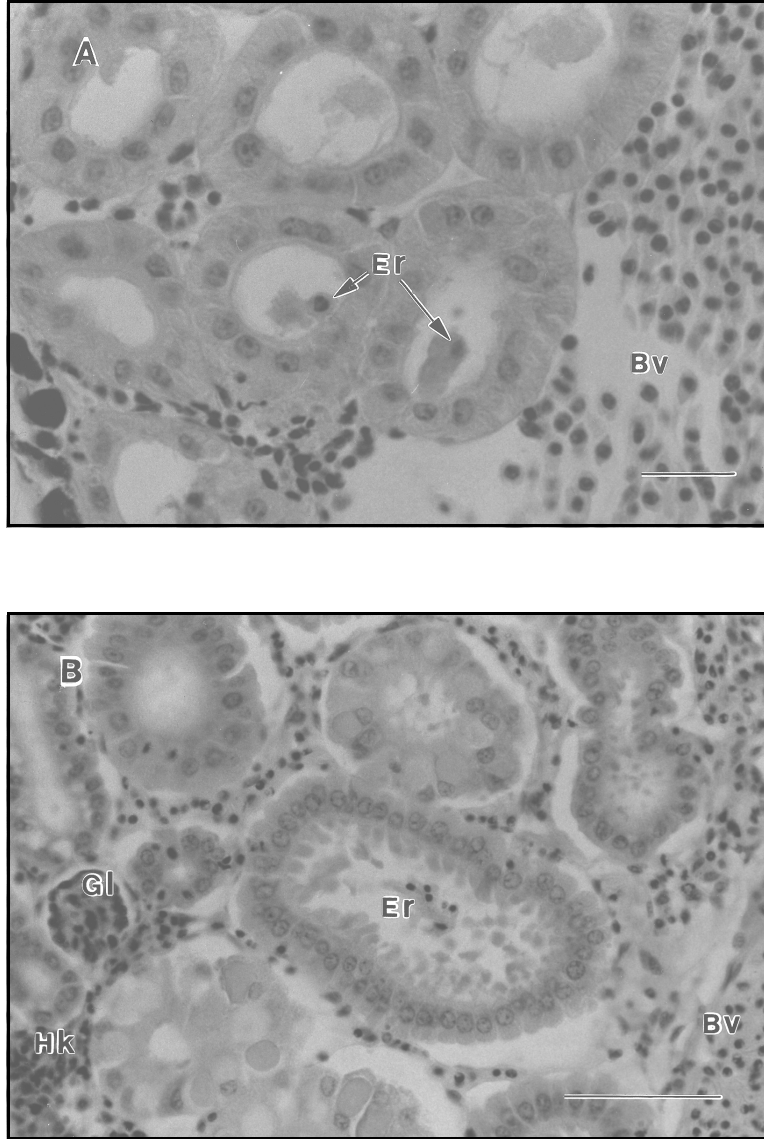


Figure 4.(A) Hematuria of the kidney of a 16.7 mm SL pinfish, *Lagodon rhomboides*, exposed to a pressure wave from a sub-marine detonation (scale bar = 18 μm). (B) Hematuria in proximal nephric tubule of the kidney of a 20.2 mm SL spot, *Leiostomus xanthurus*, exposed to a pressure wave from a sub-marine detonation (scale bar = 43 μm): Bv, blood vessel; Er, erythrocytes; Gl, glomeruli; Hk, head of the kidney.

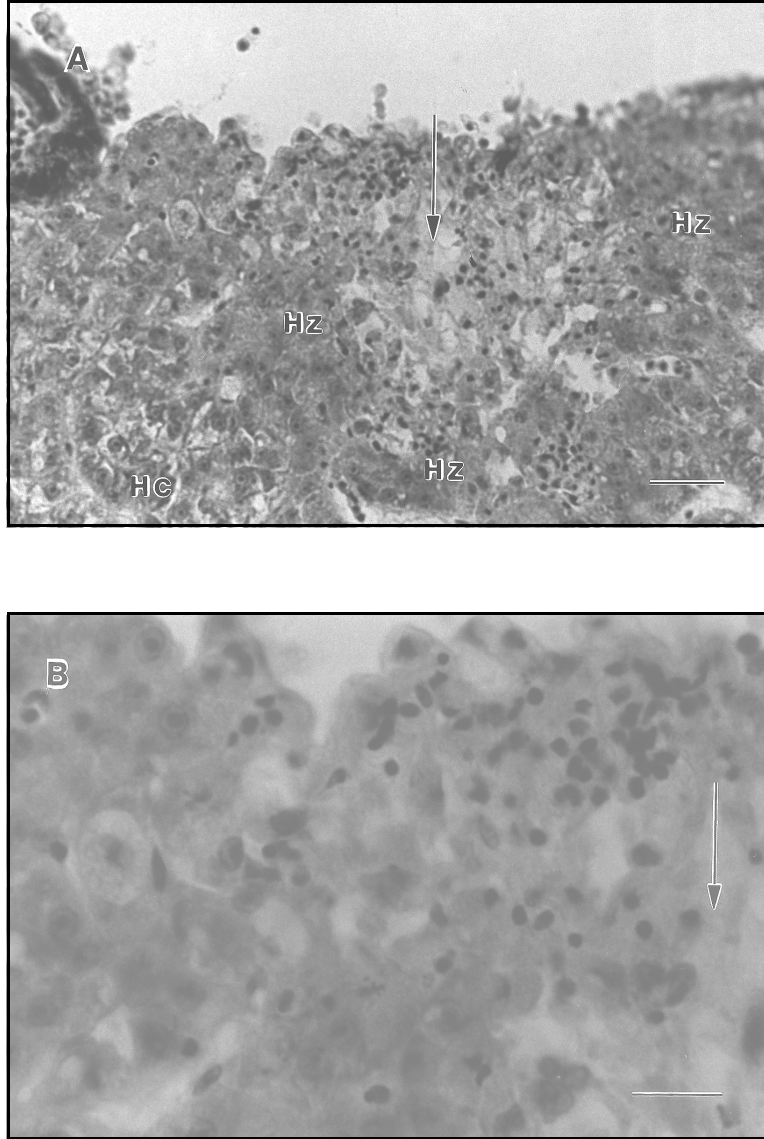


Figure 5.(A) Coagulative necrosis of the liver of a 19.7 mm SL spot, *Leiostomus xanthurus*, exposed to a pressure wave from a sub-marine detonation. The arrow indicates necrosis (scale bar = 23 μm). (B) Higher magnification of infarct. The arrow indicates necrosis (scale bar = 13 μm): Hc, normal hepatocytes; Hz, hyperemic zone.

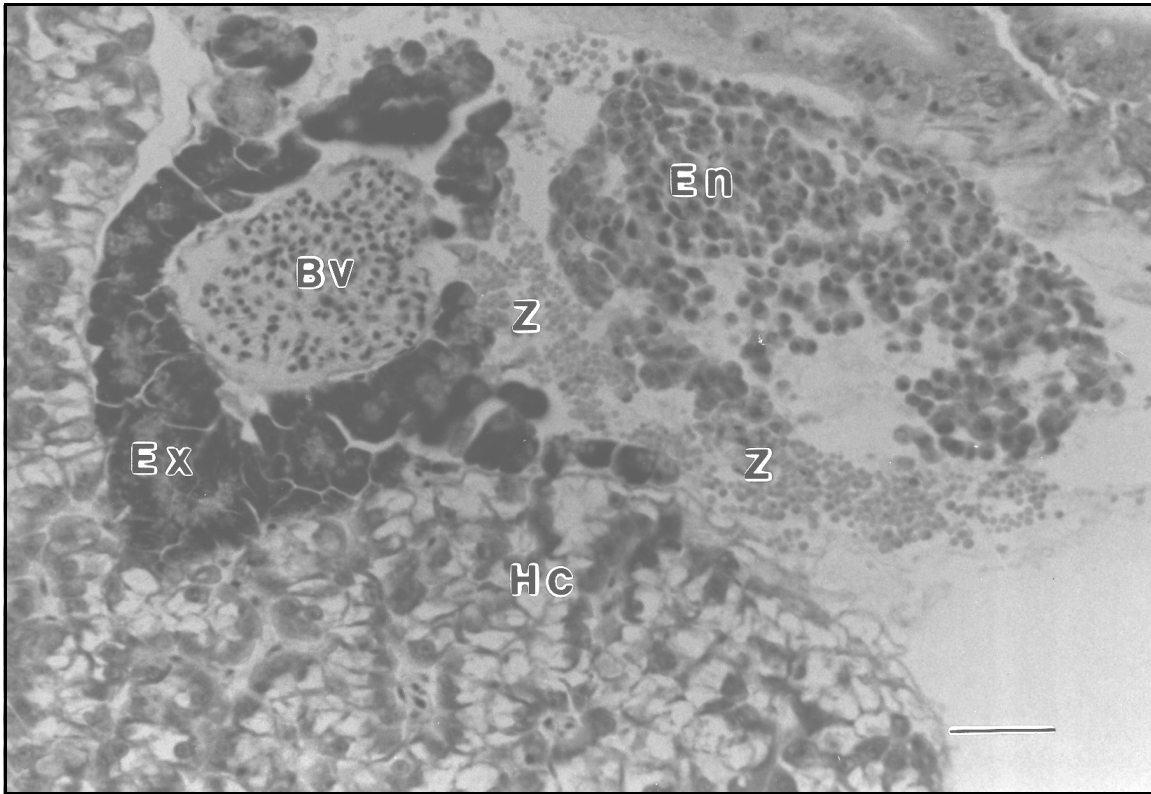


Figure 6. Rupture of the pancreas in an 18.7 mm SL spot, *Leiostomus xanthurus*, exposed to a pressure wave from a sub-marine detonation (scale bar = 30 μm): Bv, blood vessel with erythrocytes; En, endocrine pancreatic tissue; Ex, exocrine pancreatic tissue; Hc, hepatocytes; Z, zymogen granules inside and outside of the ruptured tissue.

ARA PROJECT # 0540

INSTRUMENTATION REPORT

**PRELIMINARY INVESTIGATION OF IMPACTS
OF UNDERWATER EXPLOSIONS ON
LARVAL AND EARLY JUVENILE FISHES**

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May 21, 2001

Investigation of impacts of underwater explosions on larval and early juvenile fishes.

Part 3: Instrumentation report.

Errata

Energy flux density units in this report are mJ m^{-2} and not mW m^{-2} as shown.

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INSTRUMENTATION CONFIGURATION

To better understand the effects of underwater explosions on larval fish, a series of tests were conducted on March 20 and 21, 2001 where 12-grain detonators (Primadet PDT 1403) were initiated at various distances from two types of larval fish contained in plastic bags. Underwater pressure transducers (PCB 138A01) were placed inside and outside these bags. These transducers were connected to 100-foot long RG-174 coaxial cables through waterproofed BNC connections. The cables were connected to PCB 494A21 amplifiers with their low pass filters bypassed and their excitation currents set at 20 mA. The resulting signals were recorded with Gage 512-1M digitizing units at a rate of 5 million samples per second and a resolution of 12 bits. To ensure highest quality of data, each transducer signal was recorded on two digitizing channels set at two voltage ranges. This provided a backup of the recorded signal. If a signal is voltage clipped, the channel with the greater voltage range can be used. Otherwise, the other channel is used to give better resolution.

To ensure that the digitizing system sampled during the appropriate time interval, a trigger signal was required. This was obtained by placing a wire around the detonator itself and then connecting it to a trigger box. This unit sends 10 mA through the break-wire and generates a 5-volt trigger signal when the wire is broken by the detonation. The unit incorporates optical isolation so that the common of the instrumentation system is not electrically connected to the water. The digitizing system records 1 megabyte per channel which results in approximately 100 milliseconds of recording for each channel. All channels are simultaneously sampled and utilize one eighth of the memory to record the pre-trigger data.

In addition to recording each transducer signal, the digitizing system also recorded the trigger signal, and an open channel which can be used to reduce ground-loop noise if required. The data was recorded onto the hard drive in a binary format immediately after each shot. At the end of each day, the data was backed up onto Zip disks to ensure that data was not lost in the event of a hard disk failure.

SET-UP FOR BASELINE TESTS

Prior to measurements with fish, several tests were conducted to establish the underwater pressure environment resulting from the initiation of the detonator. Initially, the detonators were placed in the channel bottom to simulate typical blasting operations. After several tests, it was determined that the resulting pressure signals were too inconsistent using this method, and that it was easy to dislodge the break-wire which results in no data collection. The location of the detonator was changed to 2 meters under the water's surface. Three shots at three different locations were conducted with one pressure transducer inside a plastic bag (same type as used to hold fish) and one without a plastic bag. Table 1 shows the configuration for each shot.

Table 1. Configuration for Shots 1 through 15.

Shot #	Date	Time	Distance	Comment
1	05-20	11:35 am	17 meters	Detonator in soil
2	05-20	12:12 pm	17 meters	Detonator in soil
3	05-20	12:32 pm	17 meters	Detonator in soil
4	05-20	12:58 pm	7.5 meters	Trigger problem
5	05-20	1:21 pm	7.5 meters	Trigger problem
6	05-20	1:53 pm	7.5 meters	Trigger problem
7	05-20	2:39 pm	7.5 meters	Detonator 2 meters below surface
8	05-20	3:05 pm	7.5 meters	Detonator 2 meters below surface
9	05-20	3:31 pm	7.5 meters	Detonator 2 meters below surface
10	05-20	4:03 pm	3.6 meters	Detonator 2 meters below surface
11	05-20	4:28 pm	3.6 meters	Detonator 2 meters below surface
12	05-20	4:45 pm	3.6 meters	Detonator 2 meters below surface
13	05-20	5:10 pm	17 meters	Detonator 2 meters below surface
14	05-20	5:28 pm	17 meters	Detonator 2 meters below surface
15	05-20	5:43 pm	17 meters	Detonator 2 meters below surface

For all of the above tests, Transducer #1 (Serial Number 6056 with calibration of 5.139 mV/psi) was placed on the port side of the boat (towards the bow) at a depth of 2 meters without a bag around it, and Transducer #2 (Serial Number 6057 with calibration of 4.978 mV/psi) was placed on the starboard side of the boat (also towards the bow) at a depth of 2 meters with a bag around it.

COMPARISON OF BASELINE PRESSURES

Figures 1 and 2 show the pressure traces for outside and inside the bag at the 17-meter distance. Figure 3 combines Figures 1 and 2 onto the same set of axes for comparison. It shows that the bag does have a measurable effect on the pressure trace which is repeatable. This effect is probably caused by the air pocket at the top of the bag that proved to be too difficult to remove, and/or air entrained in the water inside the bag.

Figures 4, 5, and 6 show the pressure traces resulting from a distance of 7.5 meters and Figures 7, 8, and 9 show the pressure traces obtained with a distance of 3.6 meters.

All of the pressure traces have been time shifted so that time zero is defined as the time of primary shock arrival at the sensor. The traces have also been vertically shifted in order to compensate for any amplifier baseline drift (in other words zero psi is not always represented as zero volts). None of the traces have been smoothed.

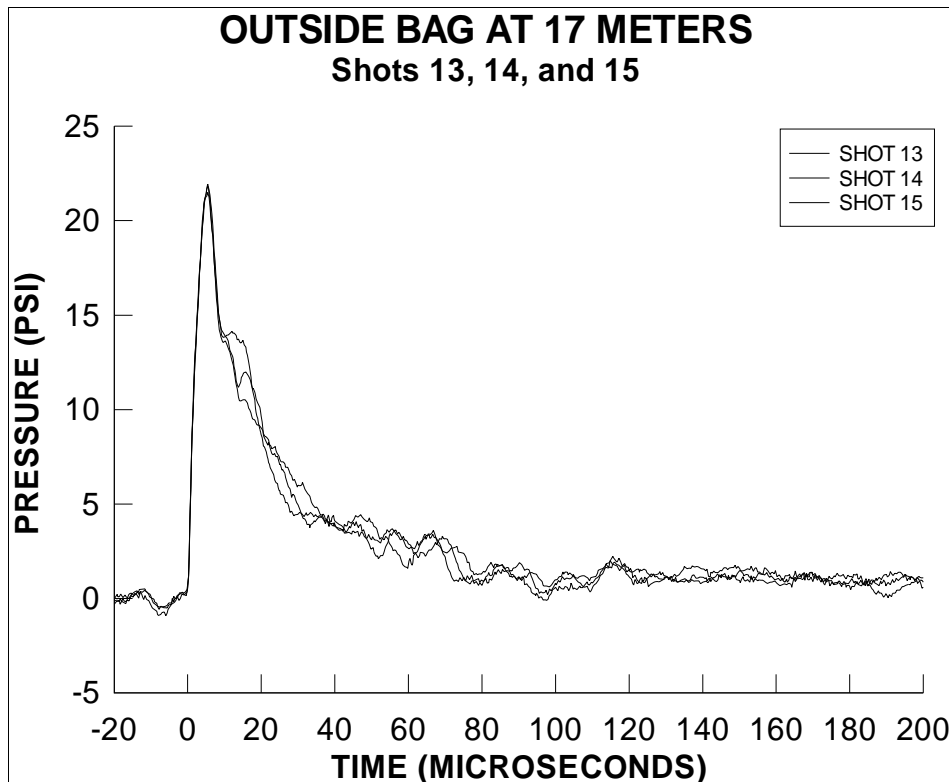


Figure 1. Pressure Traces Outside Bag at 17 meters.

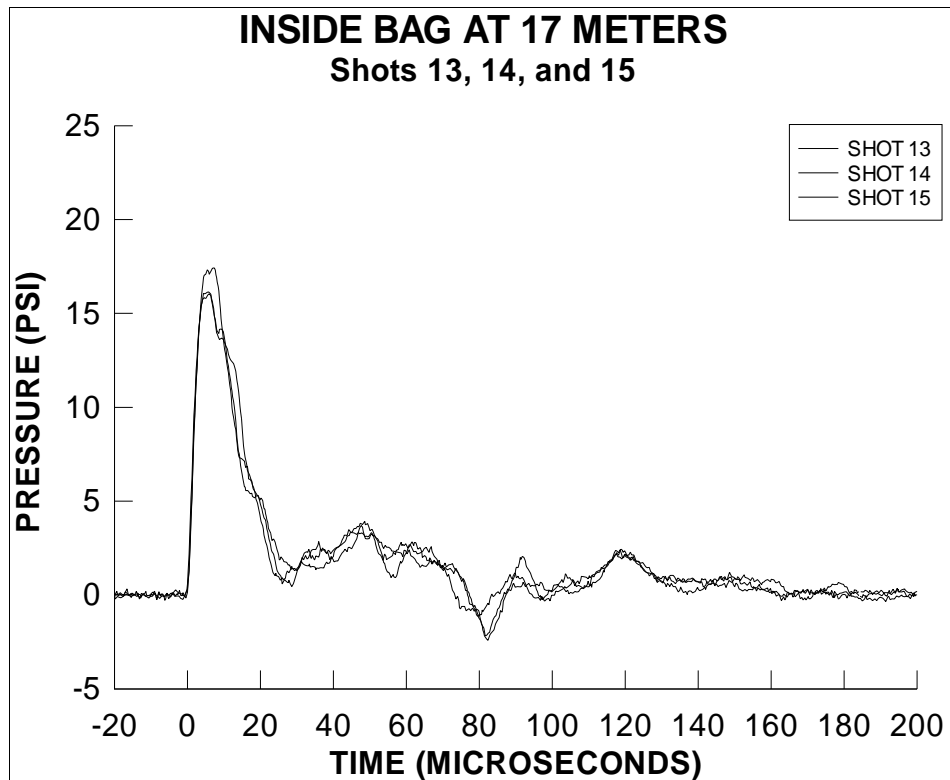


Figure 2. Pressure Traces Inside Bag at 17 meters.

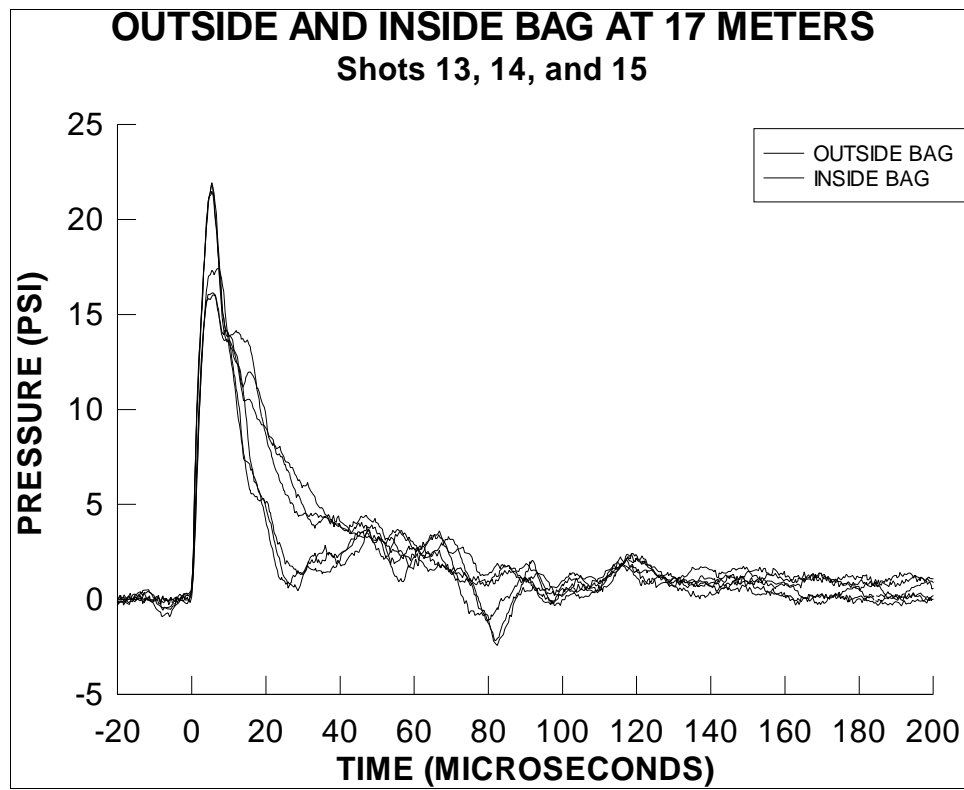


Figure 3. Pressure Traces Inside and Outside Bag at 17 meters.

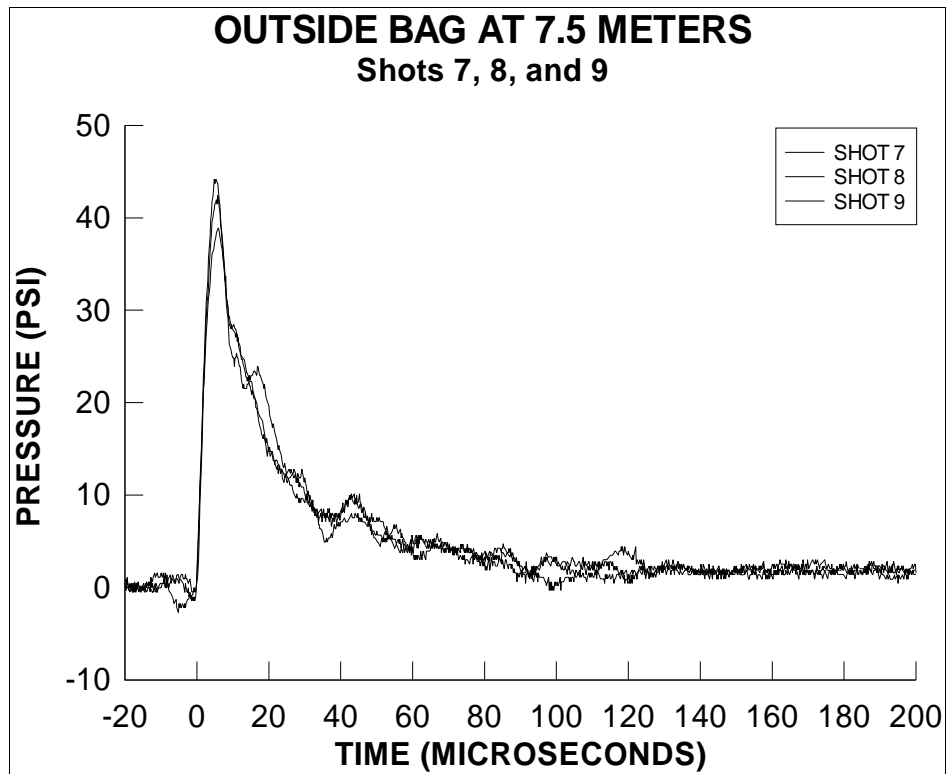


Figure 4. Pressure Traces Outside Bag at 7.5 meters.

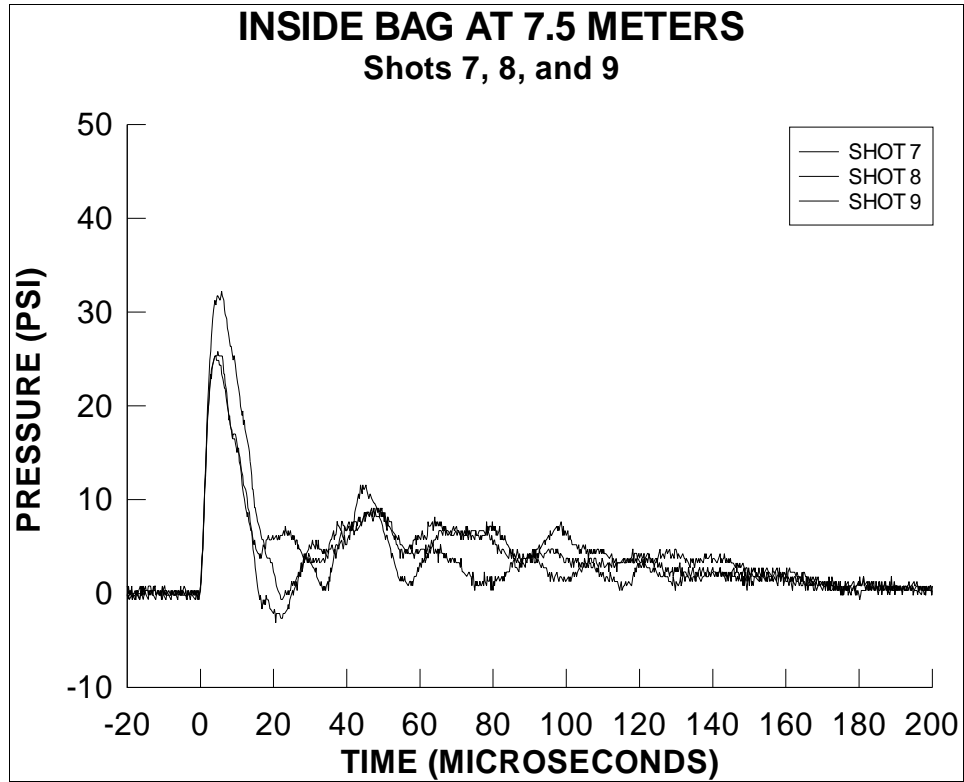


Figure 5. Pressure Traces Inside Bag at 7.5 meters.

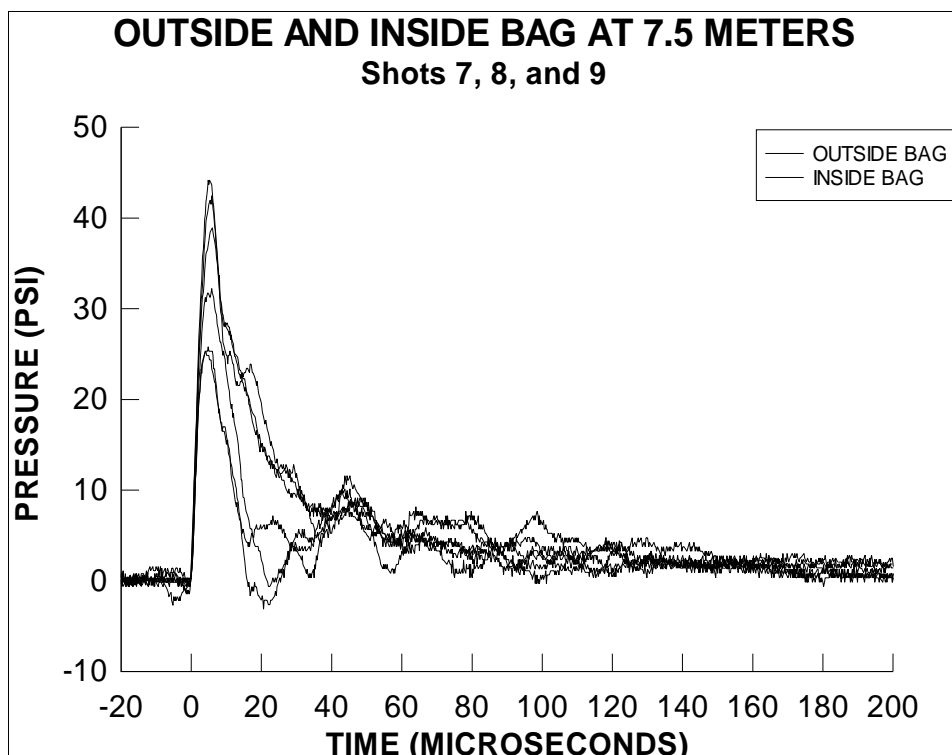


Figure 6. Pressure Traces Outside and Inside Bag at 7.5 meters.

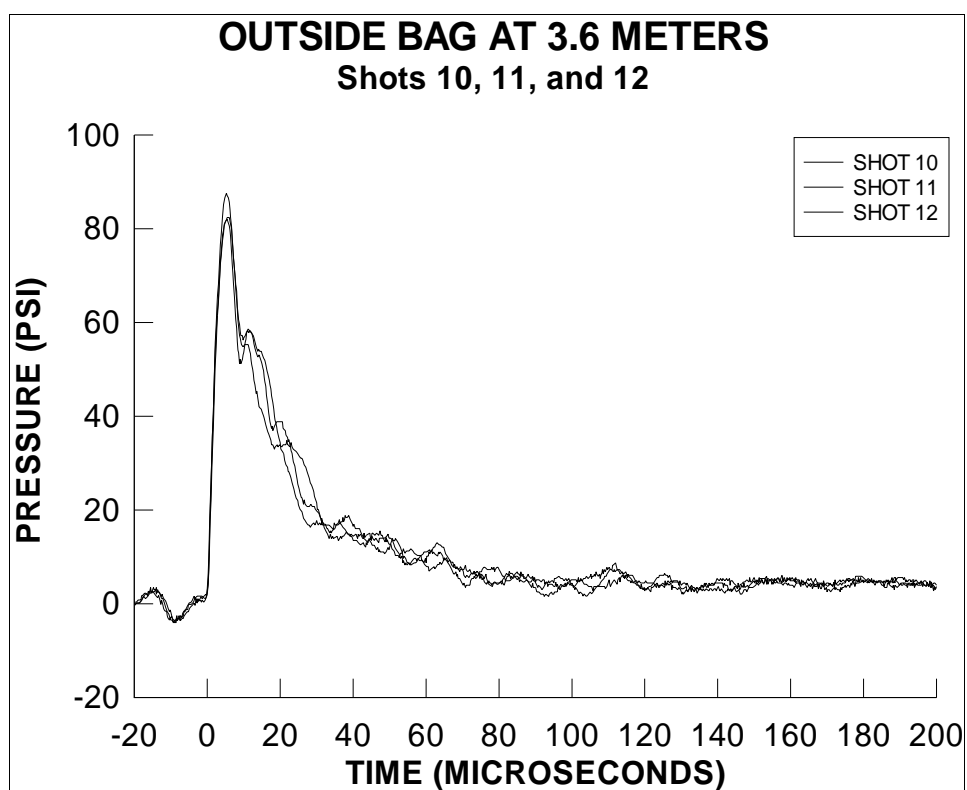


Figure 7. Pressure Traces Outside Bag at 3.6 meters.

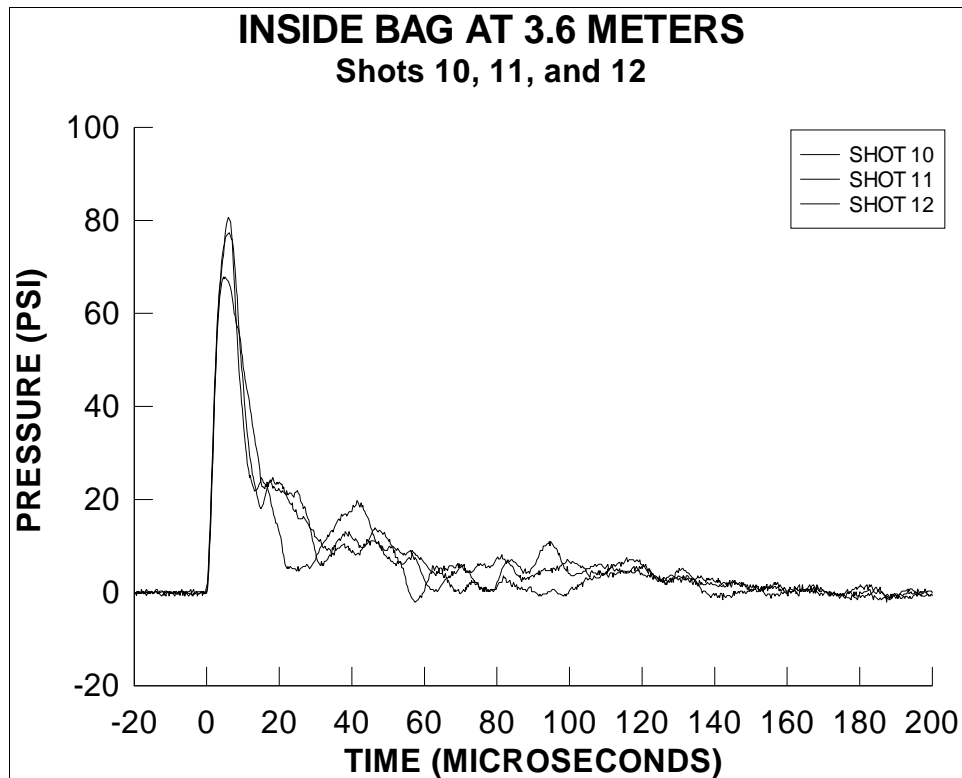


Figure 8. Pressure Traces Inside Bag at 3.6 meters.

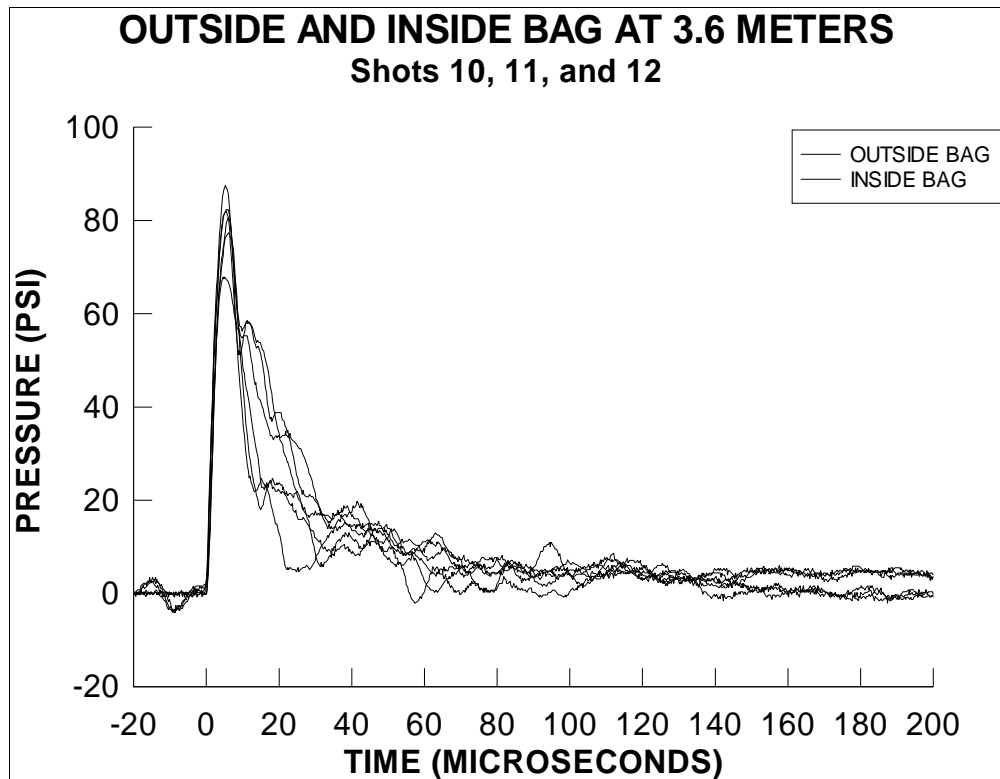


Figure 9. Pressure Traces Outside and Inside Bag at 3.6 meters.

COMPARISON OF BASELINE IMPULSES

Figure 10 shows the various impulses at the 17-meter distance as a function of time with the average pressure (outside and inside the bag) shown for reference. Figures 11 and 12 show the corresponding data for the 7.5- and 3.6-meter positions. All of these figures show that the impulses observed were very repeatable and support the assumption that the air (bubble or air entrainment) in the bag had a measurable and repeatable impact on the reading.

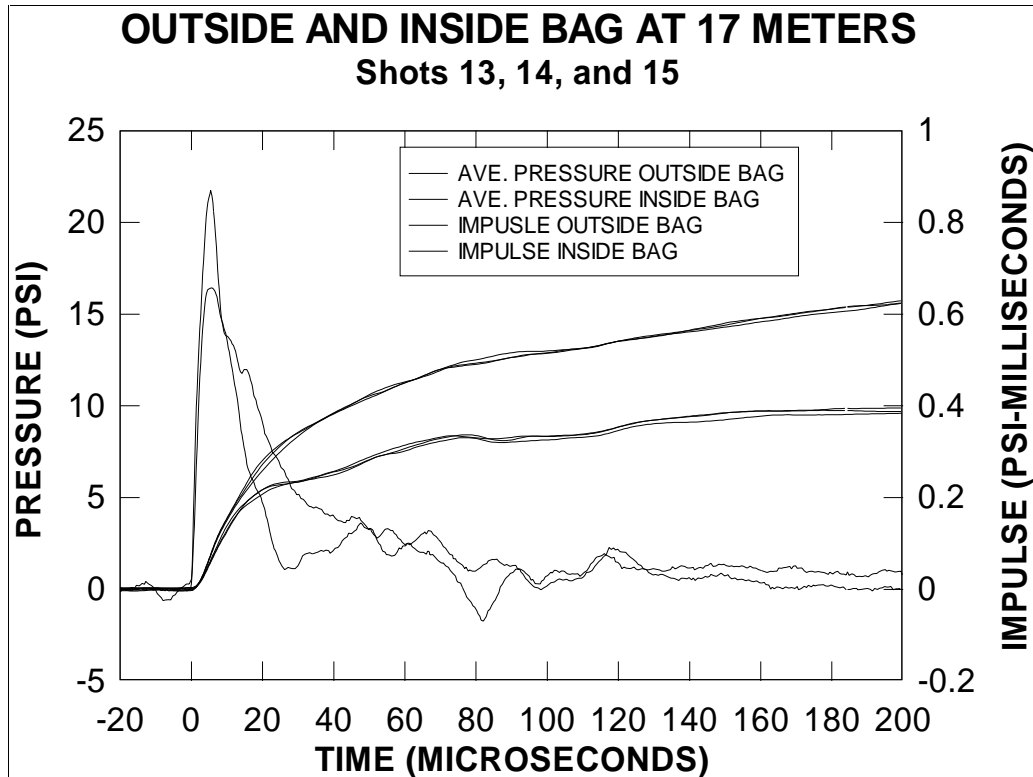


Figure 10. Impulse at 17 meters Shown as a Function of Time Compared with Pressures.

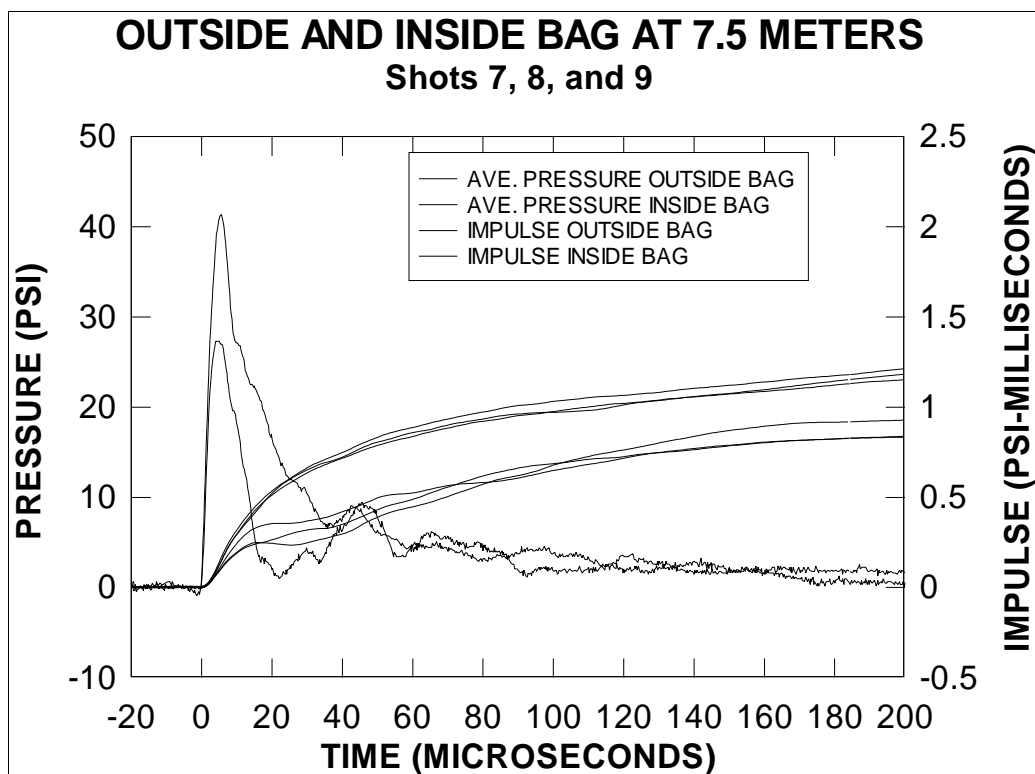


Figure 11. Impulse at 7.5 meters Shown as a Function of Time Compared with Pressures.

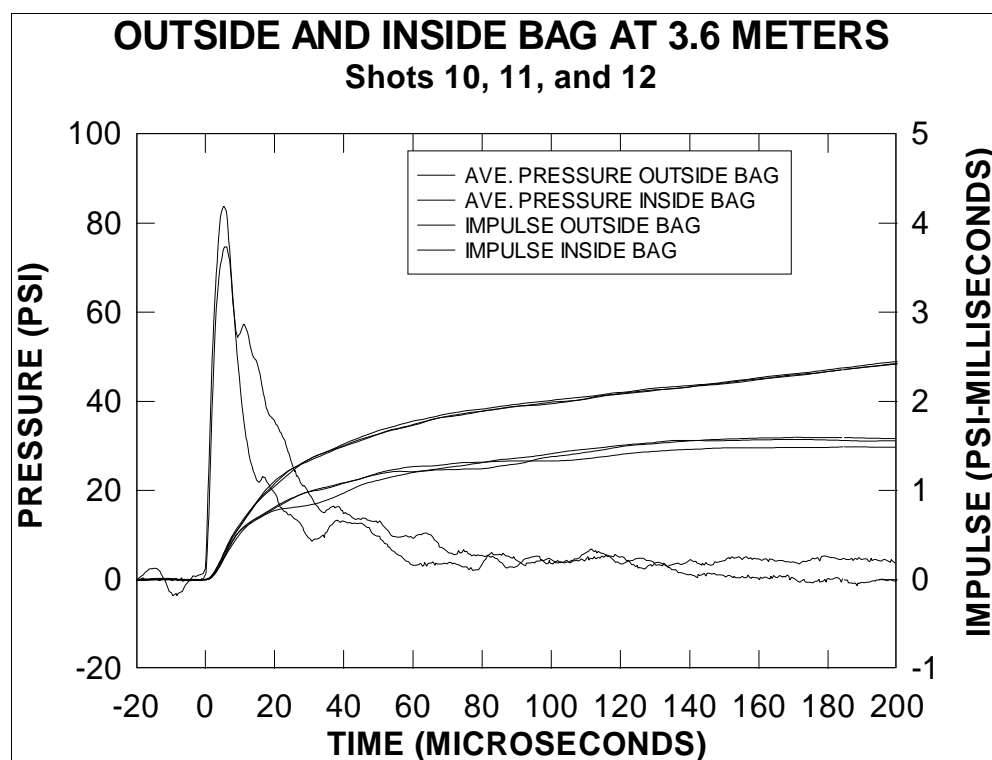


Figure 12. Impulse at 3.6 meters Shown as a Function of Time Compared with Pressures.

COMPARISON OF BASELINE ENERGY FLUX DENSITIES

Figure 13 shows the various energy flux densities (EFD) at the 17-meter distance as a function of time with the average pressure (outside and inside the bag) shown for reference. Figures 14 and 15 show the corresponding data for the 7.5- and 3.6-meter positions. With the exception of one outlier (Test 9 Transducer 2), these figures again show that the EFDs observed were very repeatable and support the assumption that the air in the bag had an measurable and repeatable impact on the reading. The water density and sonic velocity values used to create the EFD plots were collected by the Center for Coastal Fisheries and Habitat Research of the National Oceanographic and Atmospheric Administration. These values are given in Table 2.

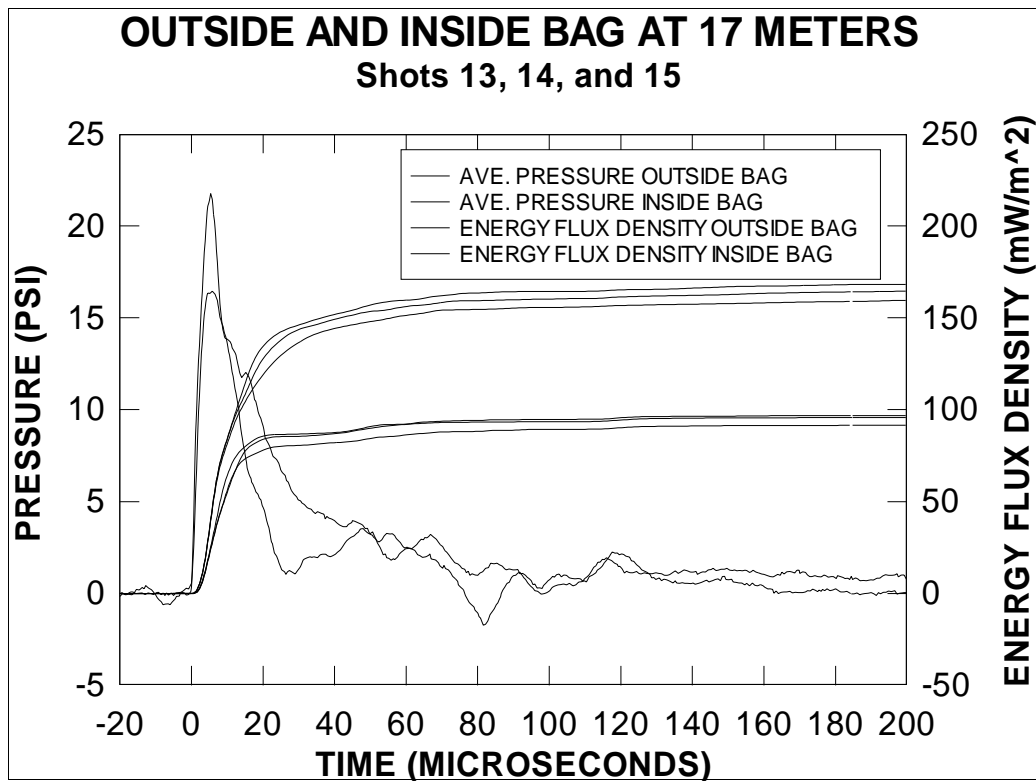


Figure 13. Energy Flux Density at 17m as a Function of Time Compared with Pressures.

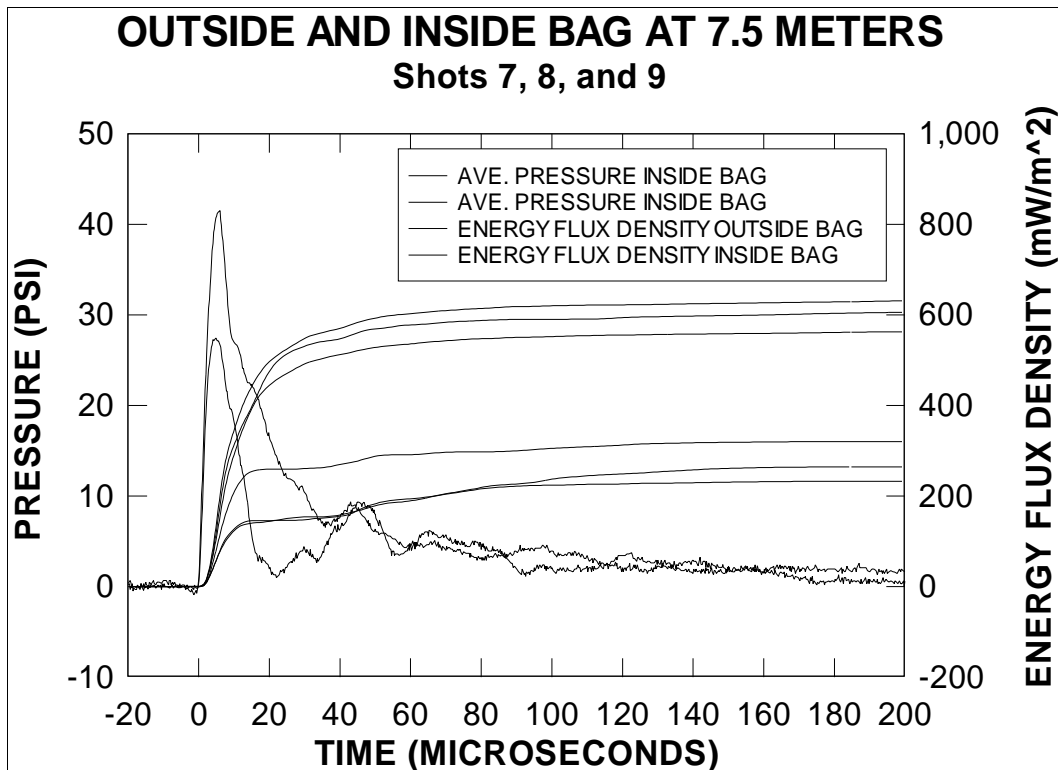


Figure 14. Energy Flux Density at 7.5m as a Function of Time Compared with Pressures.

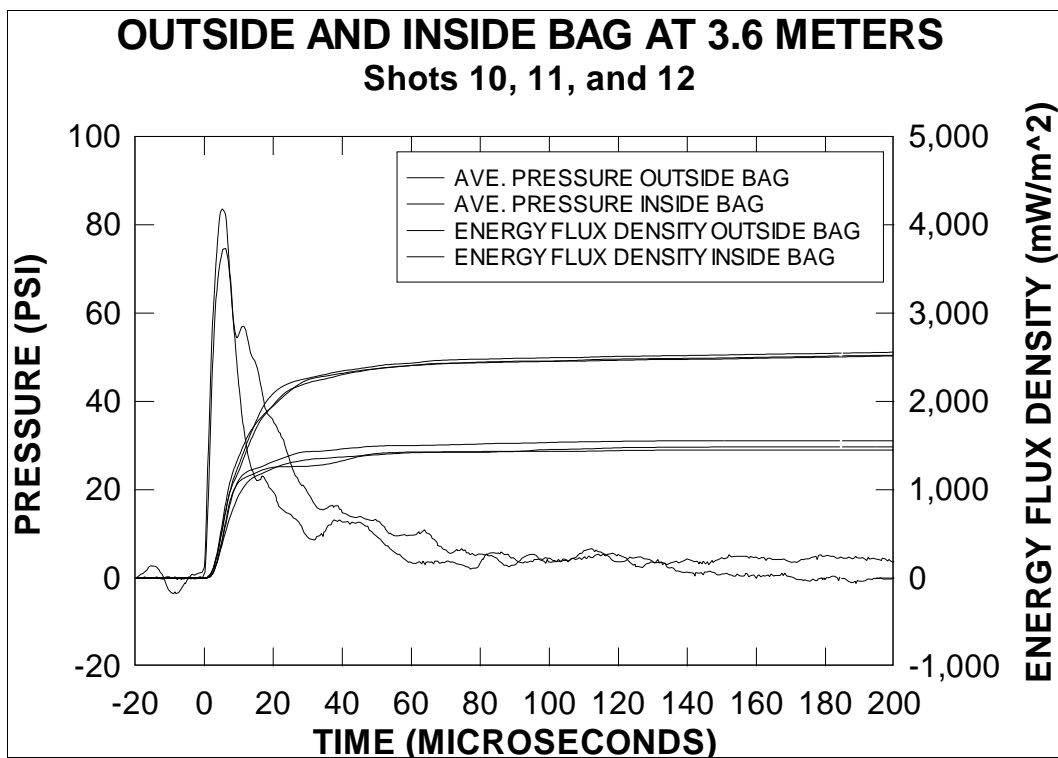


Figure 15. Energy Flux Density at 3.6m as a Function of Time Compared with Pressures.

Table 2. Water Density and Sonic Velocity for Tests 7 - 24

Test Number	Water Density (kg/m³)	Sonic Velocity (m/s)
7	1023.7	1491.9
8	1023.7	1491.9
9	1023.7	1491.9
10	1024.5	1492.6
11	1024.5	1492.6
12	1024.5	1492.6
13	1024.4	1492.5
14	1024.4	1492.5
15	1024.4	1492.5
16	1023.3	1493.5
17	1022.9	1493.0
18	1022.4	1492.6
19	1020.1	1489.5
20	1019.9	1489.6
21	1022.7	1494.6
22	1022.2	1494.1
23	1022.7	1494.1
24	1023.2	1495.0

BASELINE TEST RESULTS

Table 3 shows the resulting peak pressure, impulse, and EFD for each test and transducer. The peak was taken to be the maximum signal of the primary shock wave. The impulse was measured from time-of-arrival to 75 microseconds afterwards. This interval was chosen because it is approximately 3 time decay constants in duration which accounts for 95% of the total impulse. The EFD was obtained over the same interval as the impulse.

Table 3. Peak, Impulse, and Energy Flux Density for Shots without Fish.

Shot #	Xducer	Peak Pressure (psi)	Impulse (psi-ms)	Energy Flux Density (mW/m ²)
7	1	44.2	0.956	612
7	2	25.8	0.539	213
8	1	42.4	0.919	586
8	2	25.3	0.588	210
9	1	38.9	0.906	546
9	2	32.2	0.576	297
10	1	82.4	1.865	2475
10	2	80.6	1.240	1474
11	1	82.0	1.892	2435
11	2	77.4	1.283	1513
12	1	87.6	1.853	2437
12	2	67.9	1.313	1433
13	1	21.5	0.486	154
13	2	16.0	0.336	88
14	1	21.8	0.488	159
14	2	17.5	0.330	94
15	1	22.0	0.493	163
15	2	16.2	0.334	93

Figure 16 shows how the signal is impacted by reflections from various surfaces in the testing environment. The time scale zero is referenced to the time of detonator initiation. It takes approximately 10.3 milliseconds for the primary shock wave to travel from the detonator to the transducer. The shock wave traveling from the detonator to the water's surface and back down to the transducer arrives at 10.6 milliseconds. It is inverted because the acoustic impedance of the air is less than that of the water. At 12.2 milliseconds, two shock waves arrive virtually simultaneously which correspond to the reflection off of the bottom of the boat and the reflection from the bottom of the channel. The shock wave reflecting off the bottom and then reflecting off the water's surface before reaching the transducer arrives at 13.7 milliseconds (and is inverted).

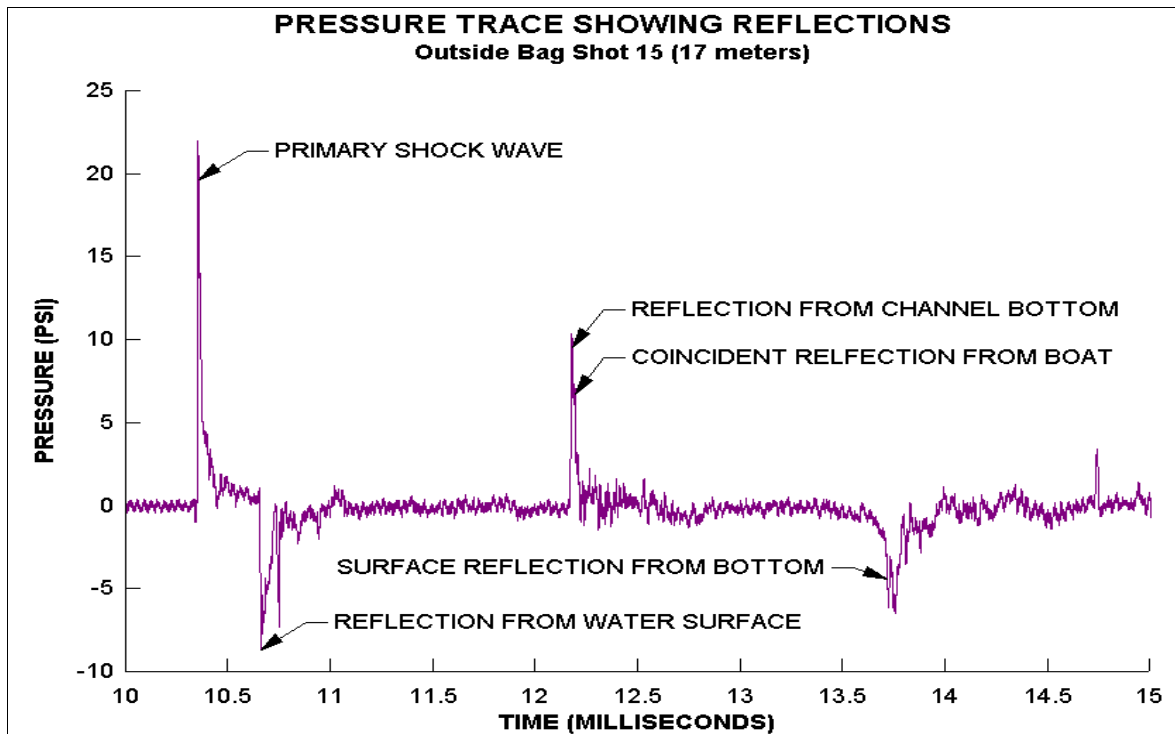


Figure 16. Example Showing Various Reflections.

SET-UP FOR TESTS WITH FISH

The tests with the fish were performed at the same distances and depths as the previous baseline tests. Two bags of fish were used in each test (one type on the port and the other type on the starboard) with a transducer placed inside each bag. Table 4 shows the configuration for these tests.

Table 4. Configuration for Shots 16 through 24.

Shot #	Date	Time	Distance
16	05-21	9:38 am	3.6 meters
17	05-21	10:08 am	3.6 meters
18	05-21	10:36 am	3.6 meters
19	05-21	11:46 am	7.5 meters
20	05-21	12:29 pm	7.5 meters
21	05-21	2:45 pm	7.5 meters
22	05-21	4:08 pm	17 meters
23	05-21	4:43 pm	17 meters
24	05-21	5:22 pm	17 meters

For all of the above tests, Transducer #1 (Serial Number 6056 with calibration of 5.139 mV/psi) was placed on the port side of the boat (towards the bow) at a depth of 2 meters inside a bag with one species of fish, while Transducer #2 (Serial Number 6057 with calibration of 4.978 mV/psi) was placed on the starboard side of the boat (also towards the bow) at a depth of 2 meters inside a bag with another species of fish.

PRESSURE DATA FROM TESTS WITH FISH

Figure 17 shows the pressure traces for all of the data collected at the 17-meter distance. Figures 18 and 19 are the corresponding traces for the 7.5 and 3.6 meter distances.

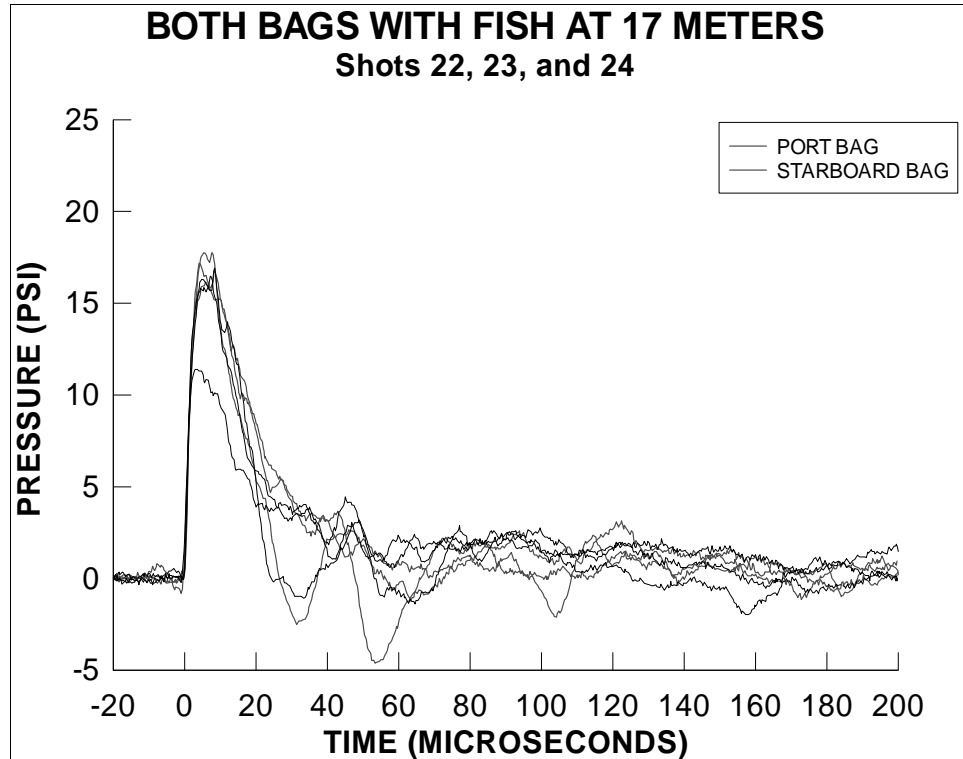


Figure 17. Pressure Traces with Fish at 17 meters.

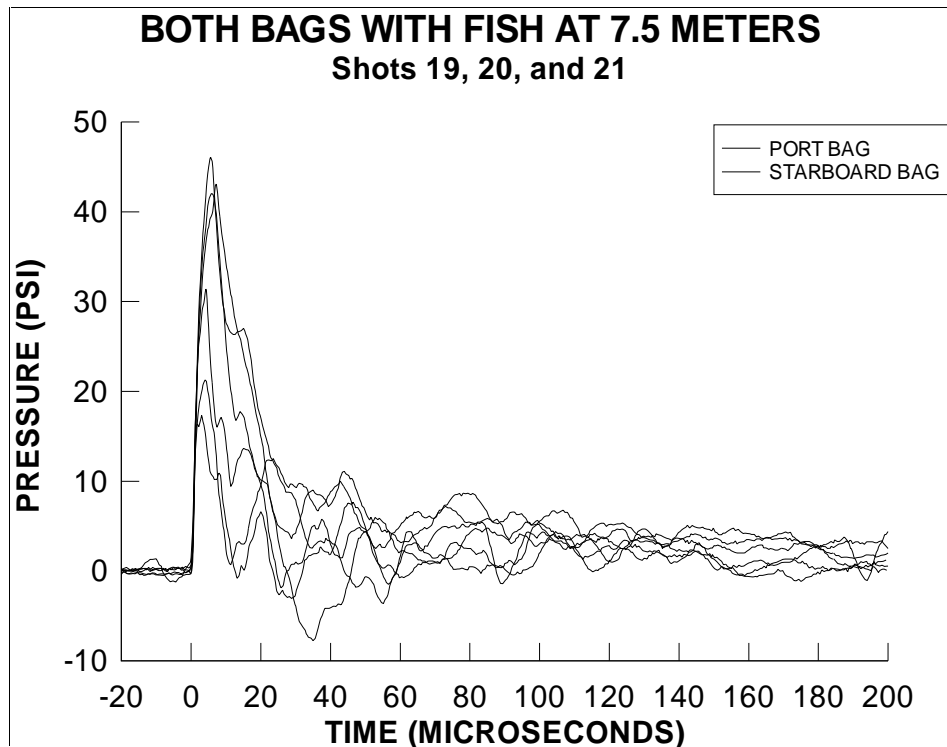


Figure 18. Pressure Traces with Fish at 7.5 meters.

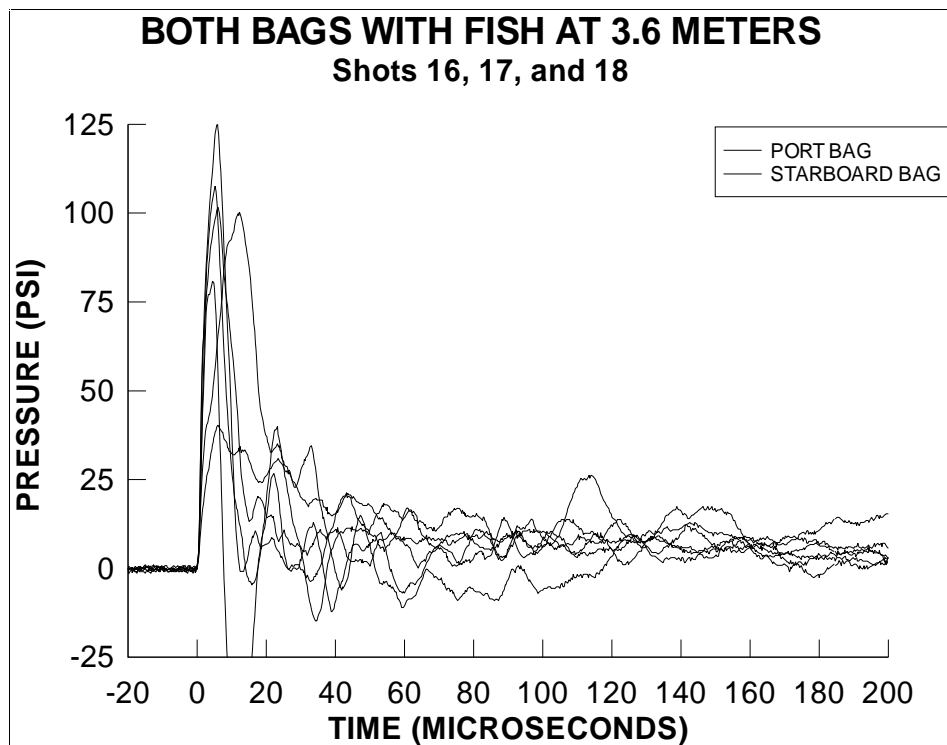


Figure 19. Pressure Traces with Fish at 3.6 meters.

RESULTS FROM TESTS WITH FISH

Table 5 shows the resulting peak pressure, impulse, and EFD for each test and transducer for the tests with fish. The peak was taken to be the maximum signal of the primary shock wave. The impulse and EFD were measured from time-of-arrival to 75 microseconds afterwards (the same as the measurements without fish).

Table 5. Peak, Impulse, and Energy Flux Density for Shots with Fish.

Shot #	Xducer	Peak Pressure (psi)	Impulse (psi-ms)	Energy Flux Density (mW/m ²)
16	1	80.9	0.389	1311
16	2	100.3	1.752	3642
17	1	101.6	1.387	2394
17	2	107.7	1.575	2238
18	1	125.6	1.011	2582
18	2	40.3	1.457	1096
19	1	43.1	0.559	585
19	2	31.4	0.468	211
20	1	17.3	0.269	73
20	2	21.3	0.585	182
21	1	46.1	0.740	614
21	2	42.1	0.830	505
22	1	17.2	0.360	126
22	2	17.9	0.278	106
23	1	16.2	0.322	125
23	2	11.5	0.301	58
24	1	16.5	0.327	103
24	2	16.9	0.312	108

Tables 6 through 11 show the average peak pressure, impulse, and EFD and their variability at each location and condition. Note that the peak pressure and impulse measurements inside the bag both with and without fish are approximately the same, and that they are approximately two thirds of the corresponding value outside the bag. The variability of the measurements is determined by dividing the standard deviation by the mean. Note that for all measurements, the variability with the fish is much higher than either of the two cases without the fish. This is probably due to the possibility that fish were near the sensing element creating interferences in the pressure measurement.

The observations stated in the above paragraph apply best to the impulse measurements and apply better to the peak pressure measurements than the EFD measurements. This is due to the fact that impulse is least sensitive to small changes while the EFD is the most sensitive (due to its dependence on the square of the pressure). Note that one of the EFD measurements without fish was not used in Tables 10 and 11 because it was determined to be an outlier (perhaps a “wild” fish happened to swim by the sensor which skewed the reading).

Table 6. Average Peak Pressure (psi) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	21.8	16.6	16.0
7.5 meters	41.8	27.8	33.6
3.6 meters	84.0	75.3	92.7

Table 7. Peak Pressure Variation (%) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	0.9	4.0	13.1
7.5 meters	5.3	11.3	33.1
3.6 meters	3.0	7.2	29.0

Table 8. Average Impulse (psi-ms) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	0.489	0.333	0.317
7.5 meters	0.927	0.568	0.575
3.6 meters	1.870	1.279	1.262

Table 9. Impulse Variation (%) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	0.6	0.7	7.9
7.5 meters	2.3	3.7	31.6
3.6 meters	0.9	2.3	35.7

Table 10. Average EFD (mW/m²) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	158.9	91.8	104.3
7.5 meters	581.3	211.5*	361.6
3.6 meters	2447.9	1456.4	2210.6

*outlier removed

Table 11. EFD Variation (%) at Various Distances and Conditions.

Distance	Outside Bag	Inside Bag without Fish	Inside Bag with Fish
17 meters	2.2	2.8	21.9
7.5 meters	4.7	0.7*	58.9
3.6 meters	0.8	2.7	38.2

*outlier removed

ANALYSIS OF ERROR

The signal recorded by any transducer is only an approximation to the actual parameter being measured. A thorough understanding of the errors introduced by each component of the system avoids large discrepancies between the measurement taken and the actual parameter of interest.

Due to the very short duration times of the primary pressure pulse, it is important to understand the impact of system frequency response to the measurement being taken. In theory, the pressure pulse has a virtually instantaneous rise to peak and then decays back to the baseline value in an exponential fashion (see Figure 20). Because all transducers/systems have a maximum frequency response, the peak measured will never be exactly the same as the actual peak. If the transducer/system is fast enough, then the error introduced will be minimal. The error is calculated using the frequency response of the system and the exponential decay time constant of the phenomenon of interest.

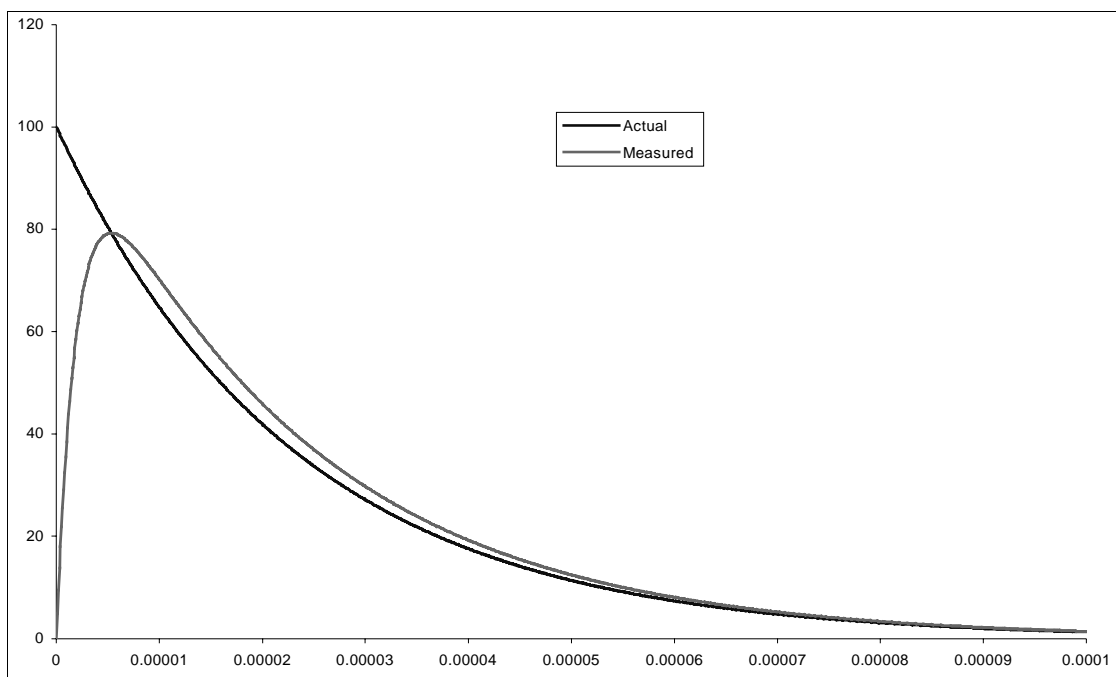


Figure 20. Example Showing Frequency Response Effects on Measurement.

Prior to field deployment, the amplifiers filters were modified for maximum frequency response, and the excitation current was set at maximum. This, in combination with the 100-foot coaxial cable used (3000 pF capacitance) gives a total system frequency response time of 1 microsecond. If the decay time of the pressure wave is taken to be 25 microseconds, then the error in the peak measurement is 13%. The peak pressure is more sensitive to the system frequency response than the impulse. For these same conditions, the error in the impulse (at 3 time constants—75 microseconds) is 0.7%. This error is small in comparison to the 5% of the impulse lost from the portion of the integral between 75 microseconds and infinity. Because the EFD is based on the square of the pressure, it is

more sensitive than the impulse which is only linearly based on pressure. At 3 time constants, the error in the EFD for the above conditions is 8.5%. The portion of the EFD lost between 75 microseconds and infinity is 0.3%. For all three measurements (peak pressure, impulse, and EFD) the measured value is always smaller than the actual value.

CONCLUSIONS AND RECOMMENDATIONS

The combination of transducers, signal conditioning, and digitizers succeeded in accurately recording the pressure versus time histories created in the underwater test environment. This is supported by the repeatability of the measurements taken and by their comparability with pre-test theoretical predictions. The most important factor in this success is the high speed response of the transducers and signal conditioning and the high sampling rate of the digitizers. The combination of underwater pressure measurements and small explosive charges creates signals that decay very rapidly which demand high speed instrumentation to accurately record them.

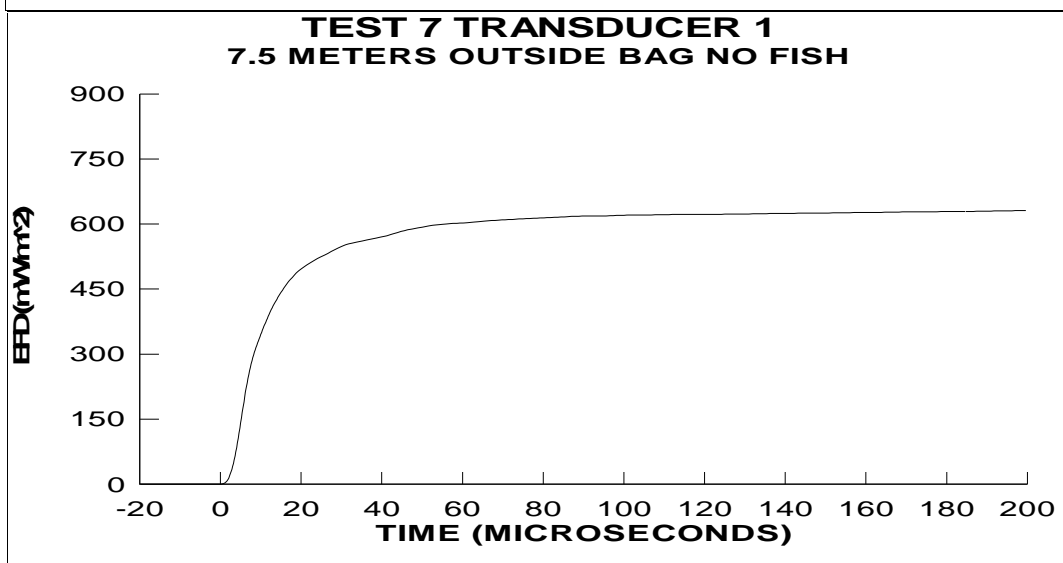
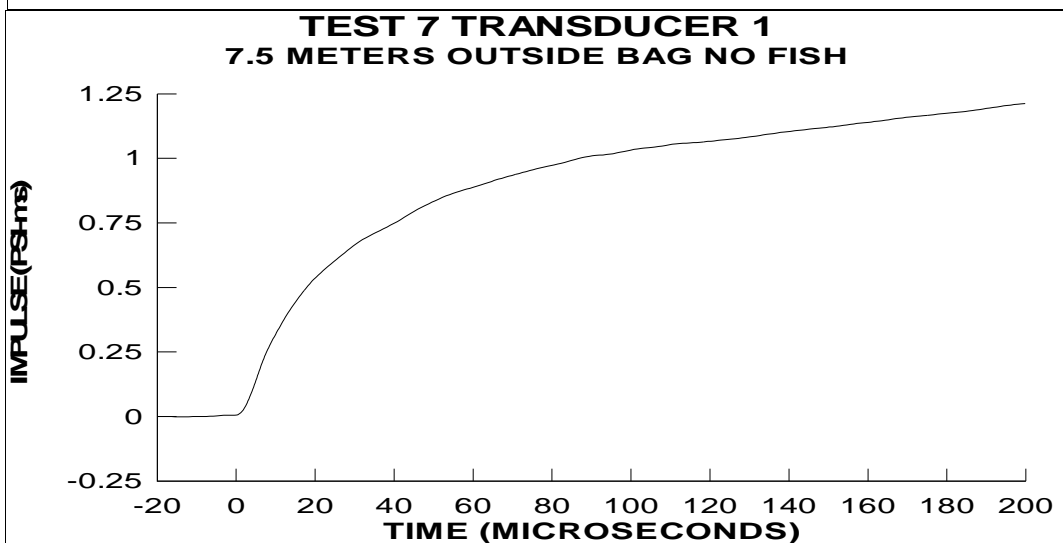
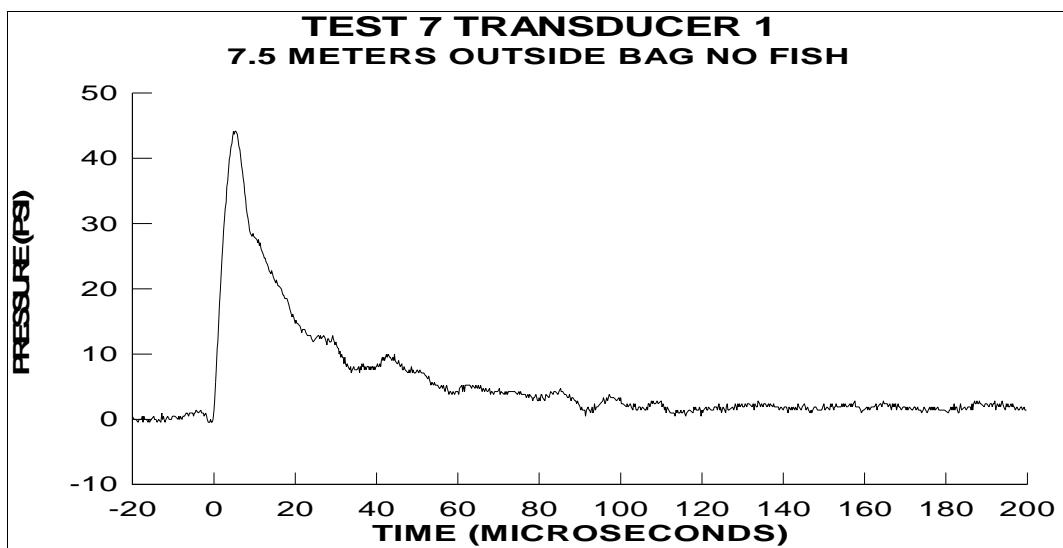
The air inside the bag and/or air entrained in the bag water had a distinct impact on the pressure measurements. It is recommended that the future tests utilize techniques to minimize the amount of air in the bag. It was determined that placing the explosive into the channel bottom introduced too much variability and was logistically more difficult than having it suspended with water on all sides. The geometry of the underwater environment did not impact the test results. Reflections from the channel bottom, water surface, and boat arrived after the primary pressure envelope was recorded.

It was demonstrated that the presence of the fish near the transducer had a significant impact on the variability of the pressure measurements, but did not seem to significantly influence the mean. In other words, in some tests, the presence of the fish reduced the reading, while in other tests their presence increased the reading.

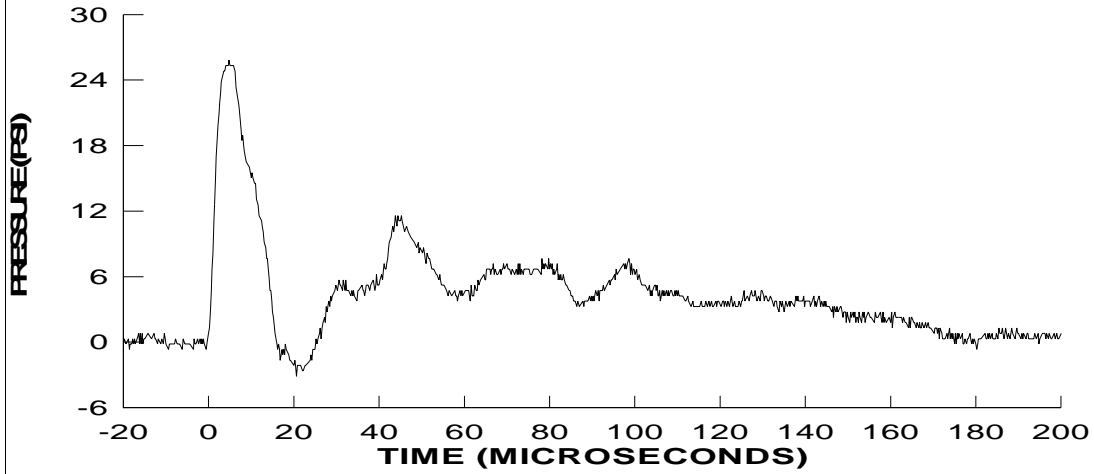
The break-wire method of triggering the digitizers worked well when the explosive was suspended in the water. However, the amount of work involved in keeping water out of the electrical circuit that it completed could be reduced by using a fiber optic method of triggering where the light generated by the explosive is transmitted down the fiber optic to a light-to-voltage conversion box. These units have successfully been deployed on numerous test events by ARA personnel.

APPENDIX

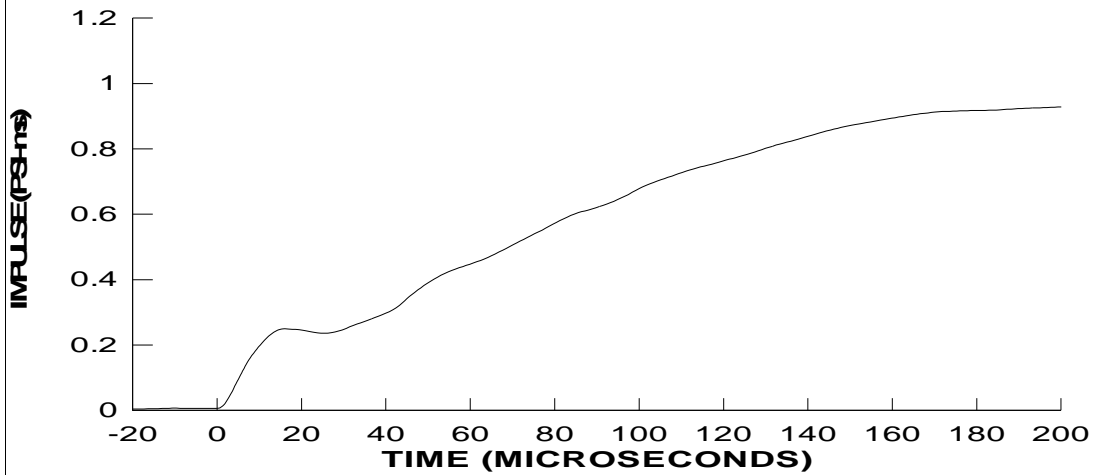
The following plots show the pressure, impulse, and EFD as a function of time. The plots have been time shifted so that time zero coincides with shock arrival. They have also been vertically shifted so that each baseline value prior to shock arrival is zero. The plots have not been smoothed.



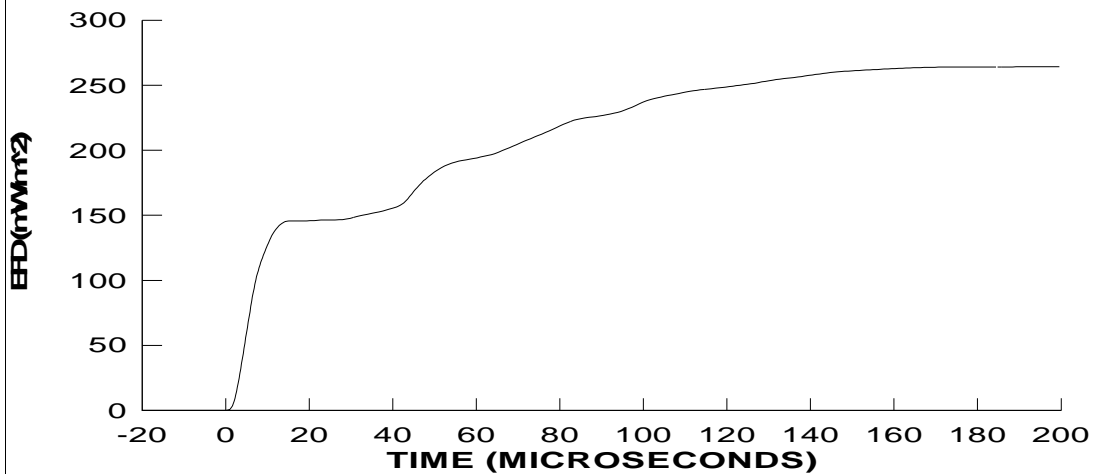
**TEST 7 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH**



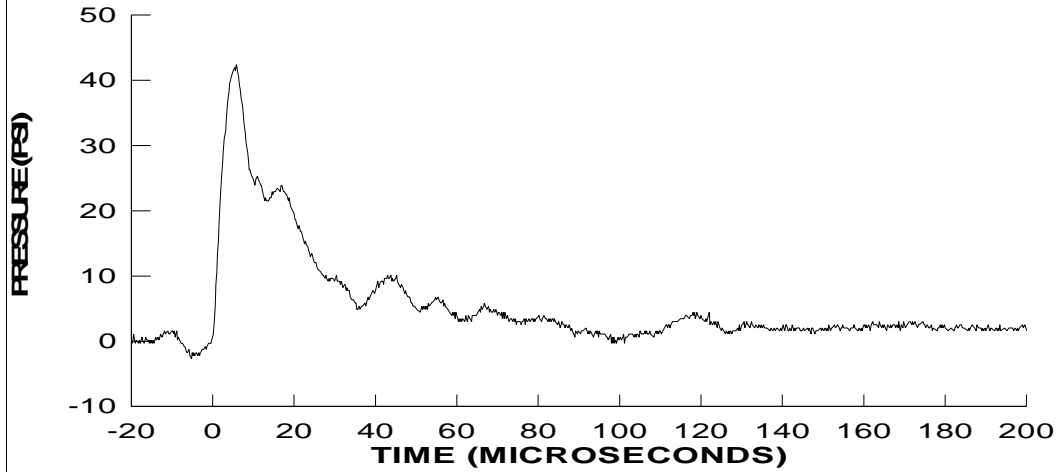
**TEST 7 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH**



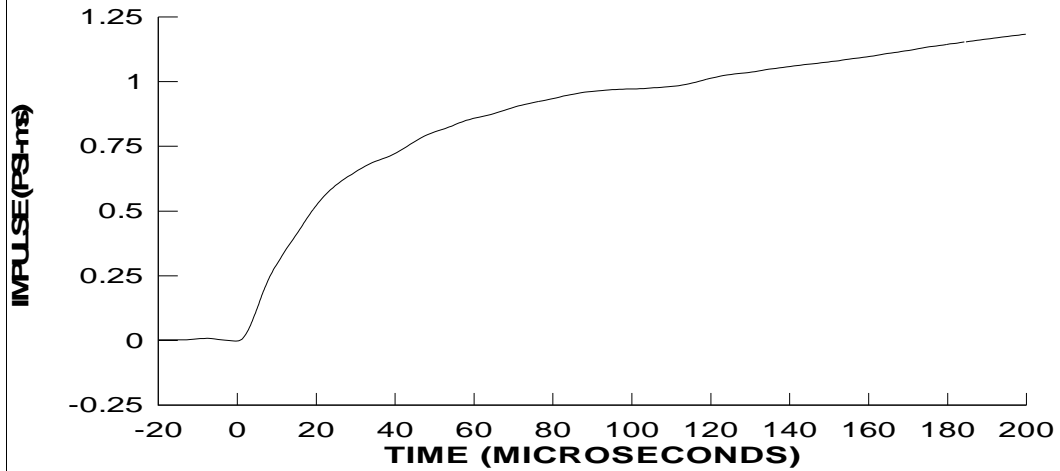
**TEST 7 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH**



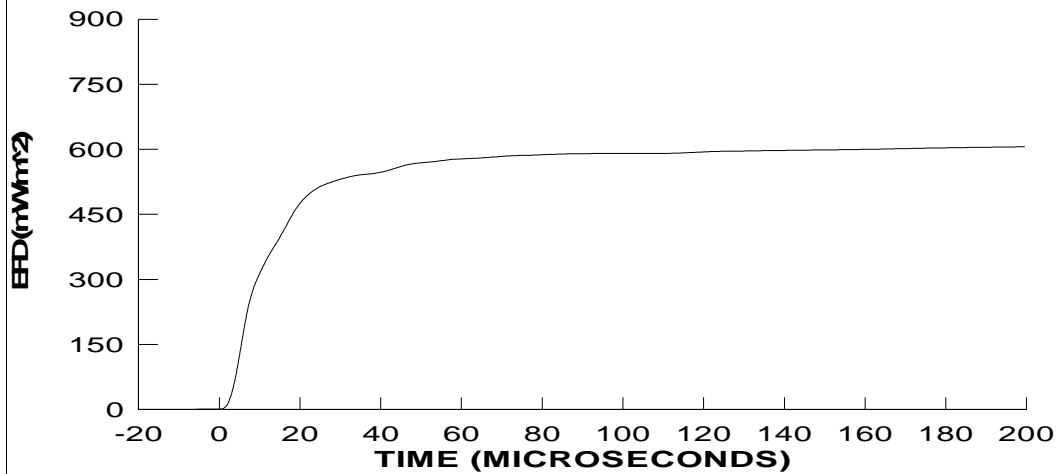
TEST 8 TRANSDUCER 1
7.5 METERS OUTSIDE BAG NO FISH



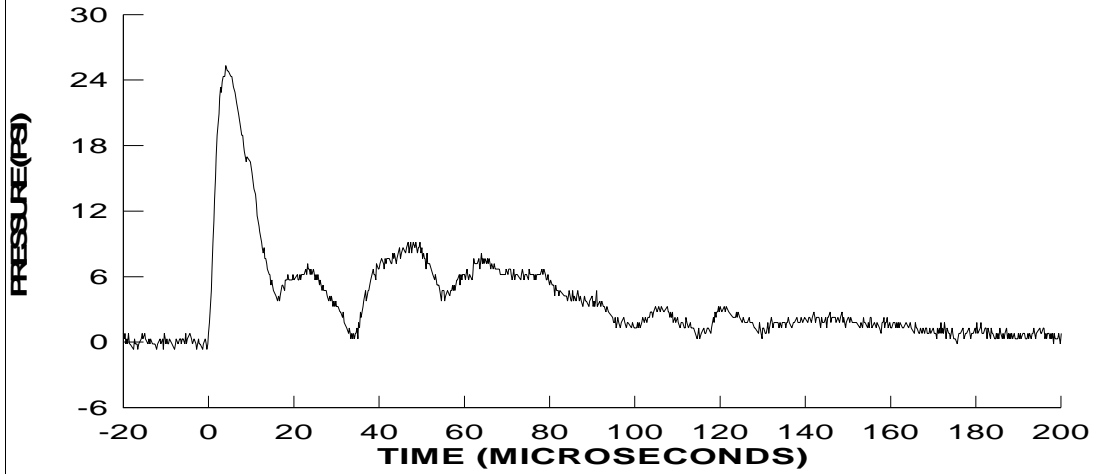
TEST 8 TRANSDUCER 1
7.5 METERS OUTSIDE BAG NO FISH



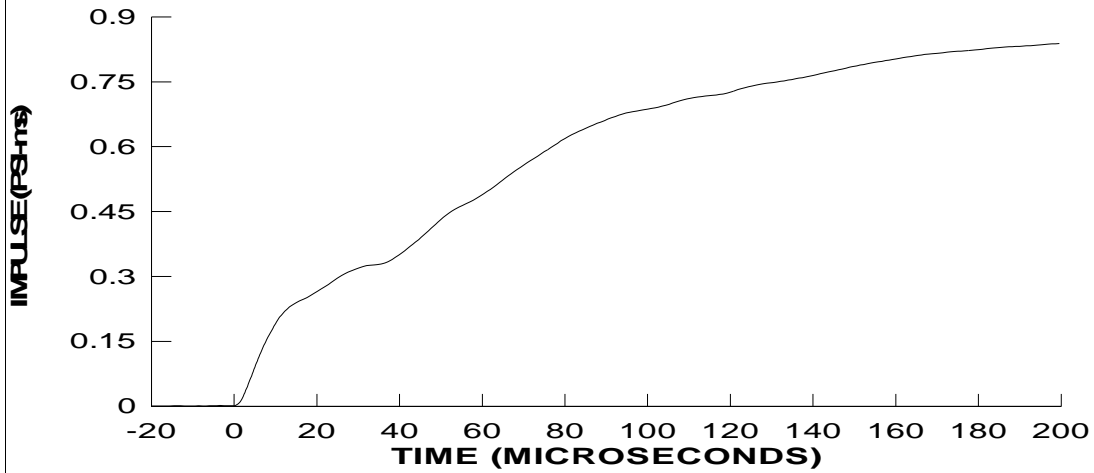
TEST 8 TRANSDUCER 1
7.5 METERS OUTSIDE BAG NO FISH



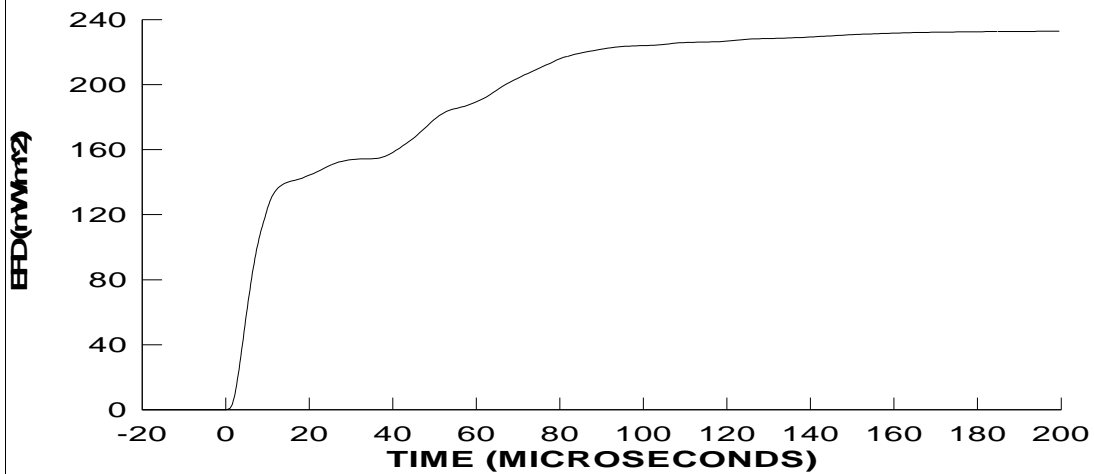
TEST 8 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH

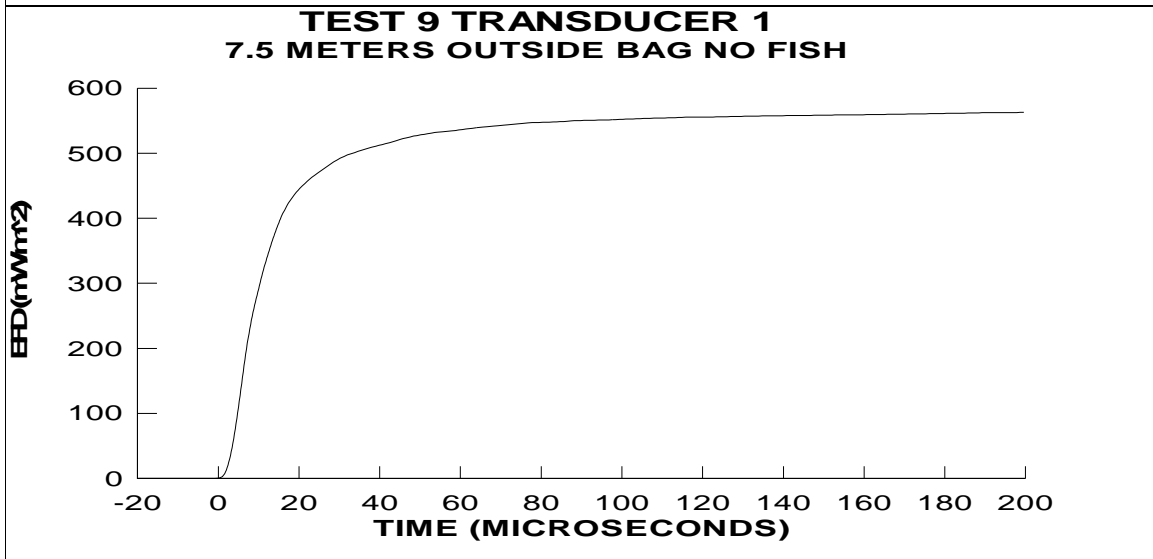
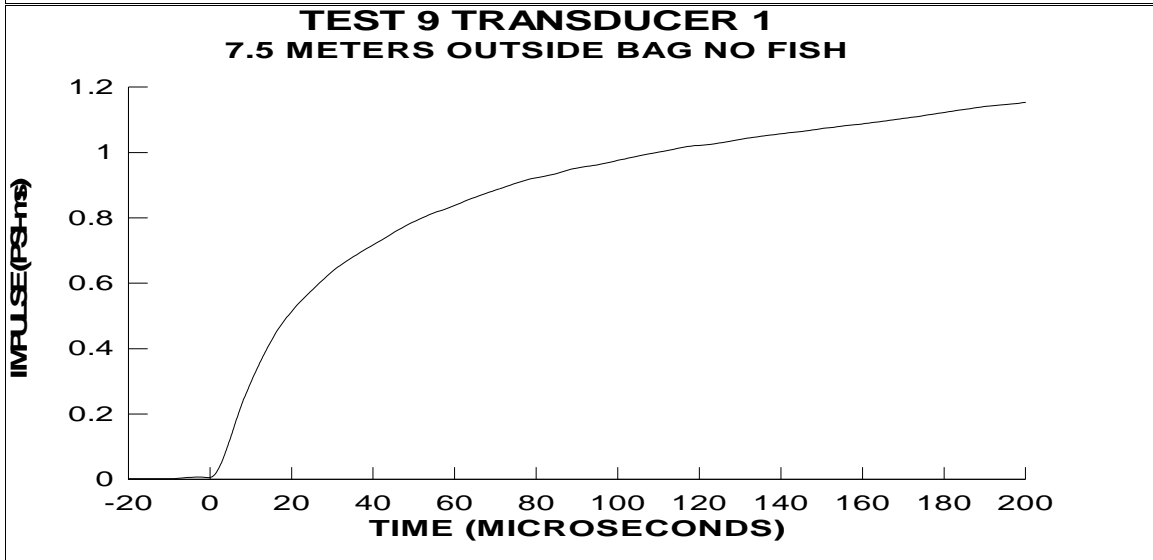
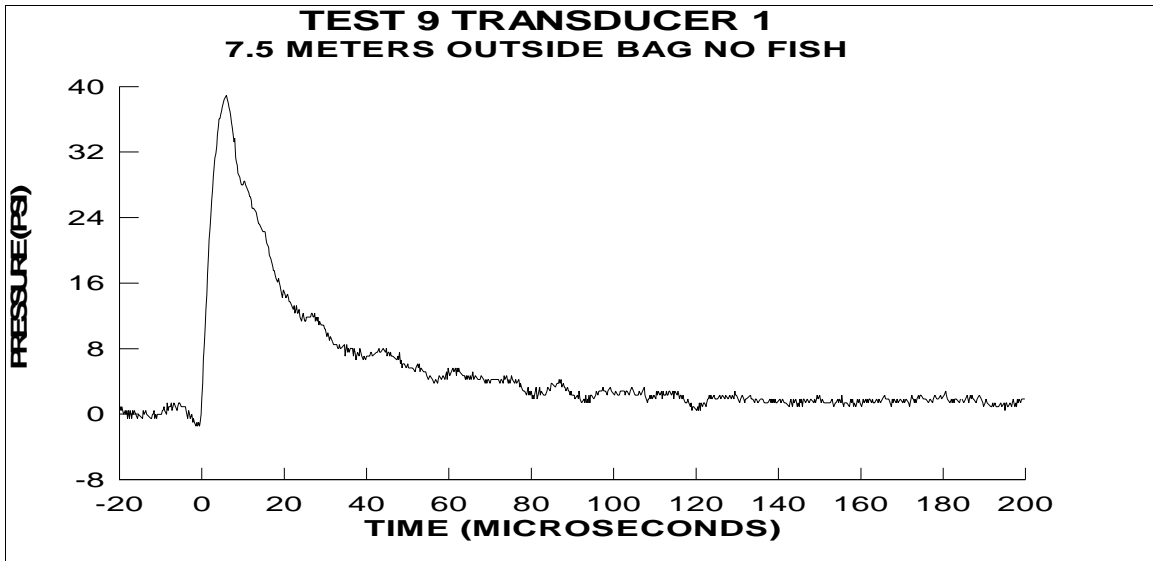


TEST 8 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH

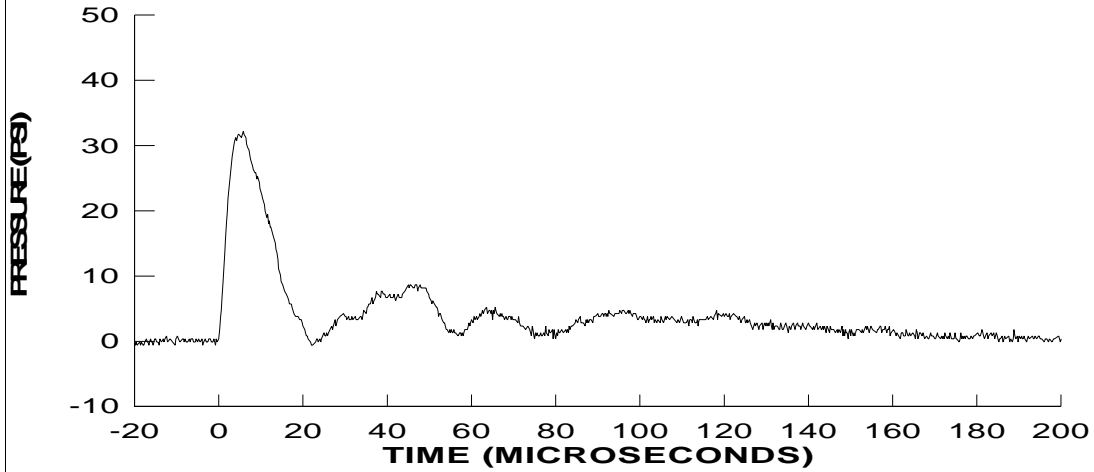


TEST 8 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH

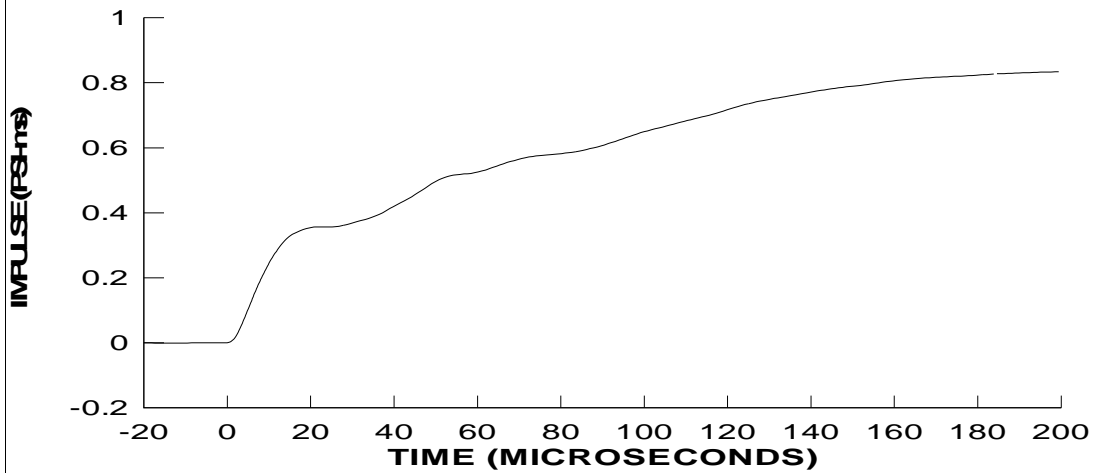




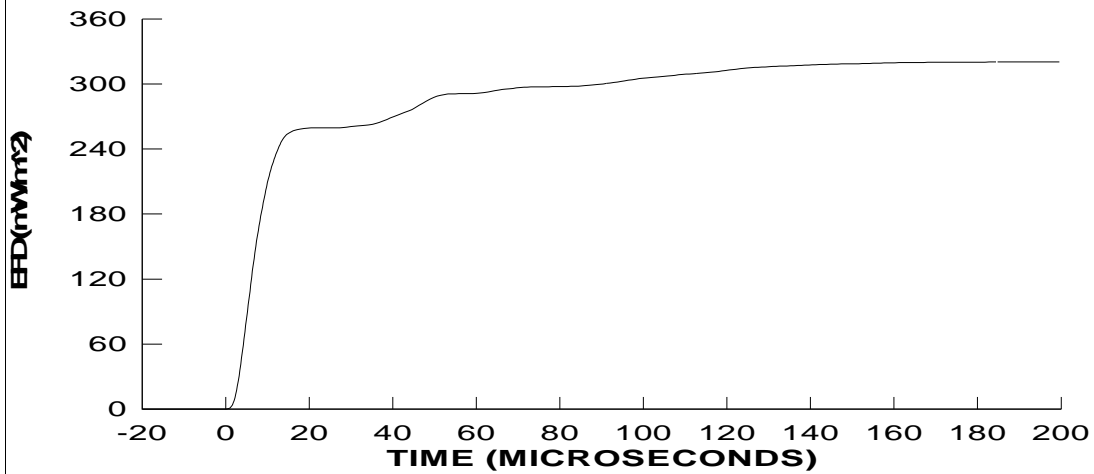
TEST 9 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH



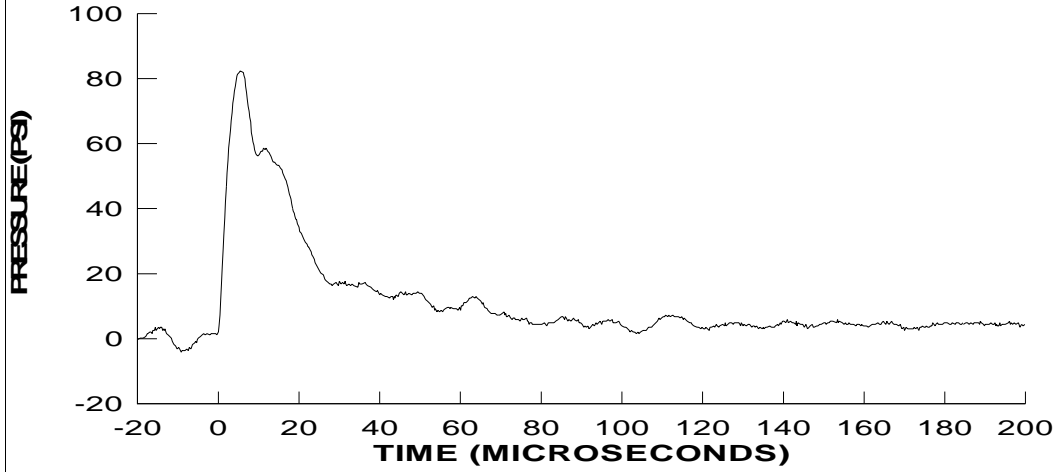
TEST 9 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH



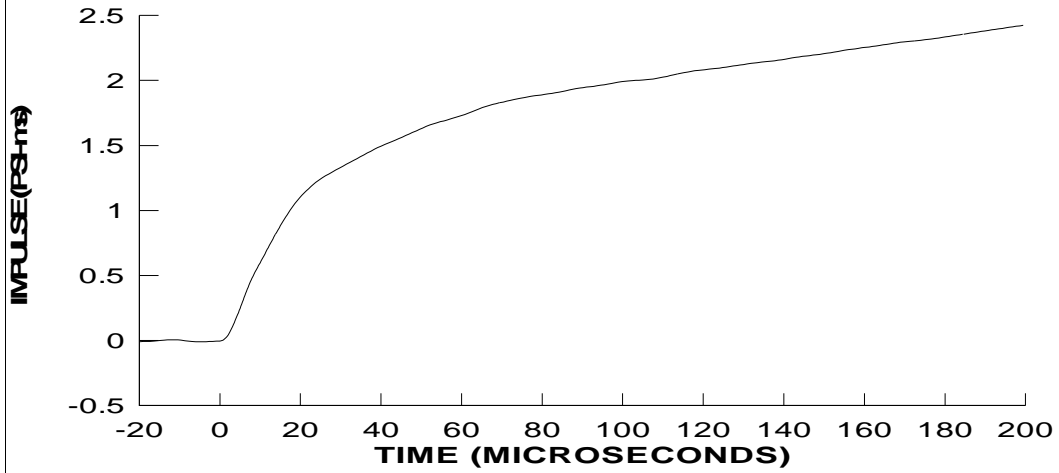
TEST 9 TRANSDUCER 2
7.5 METERS INSIDE BAG NO FISH



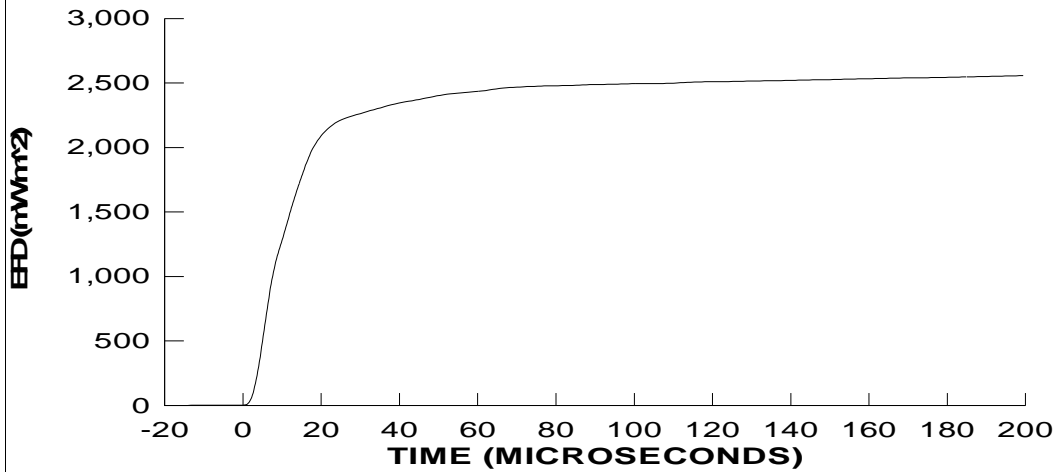
TEST 10 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH

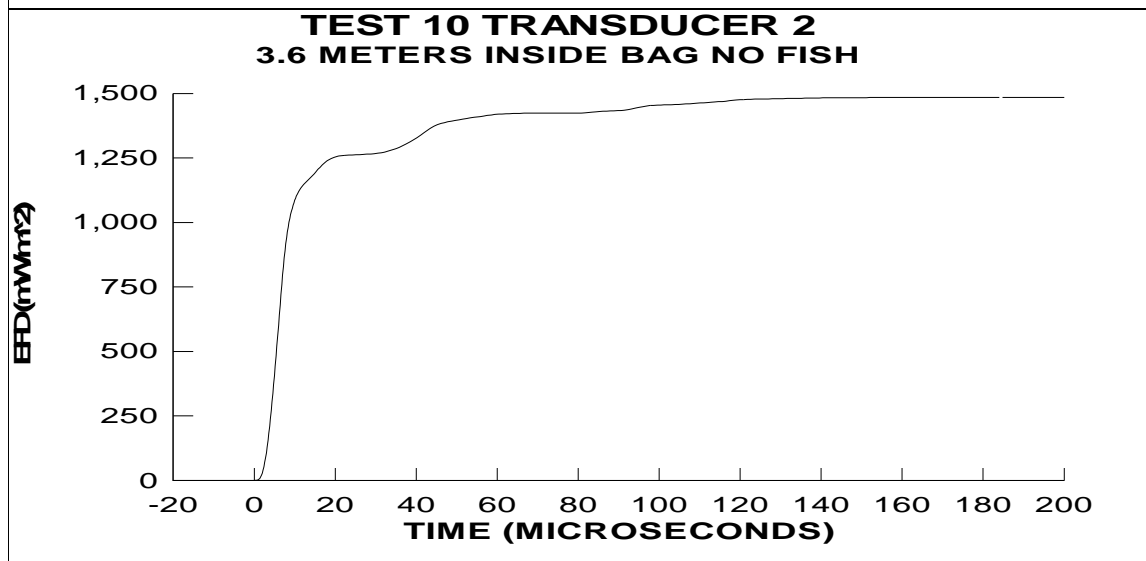
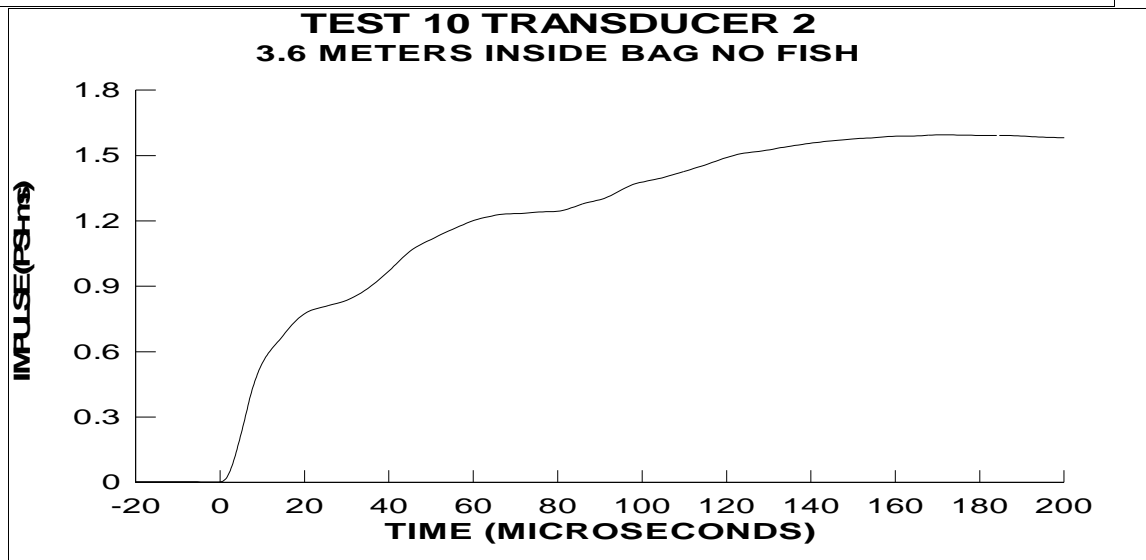
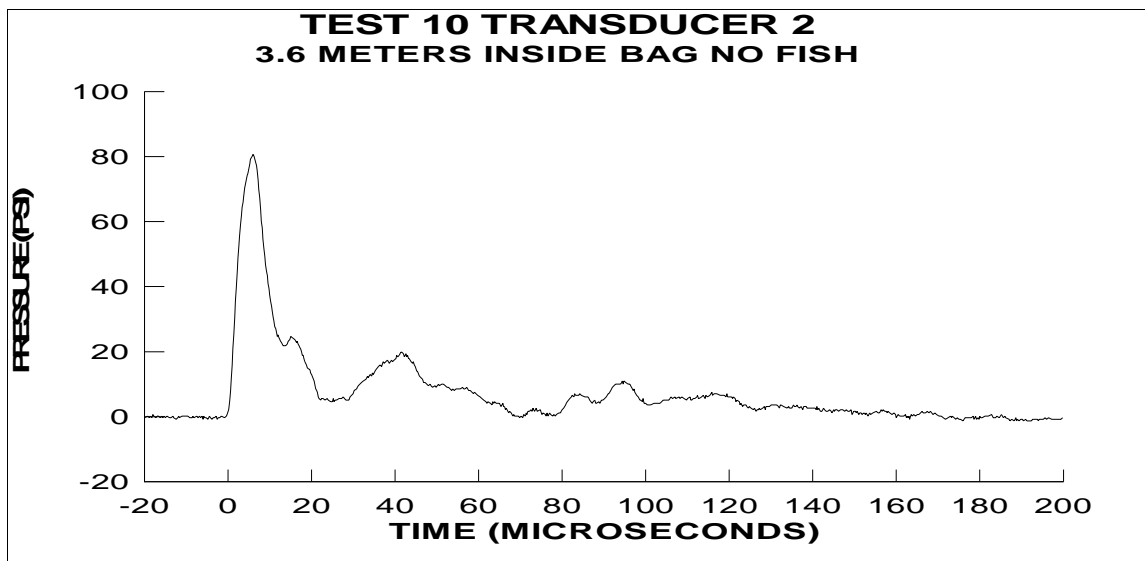


TEST 10 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH

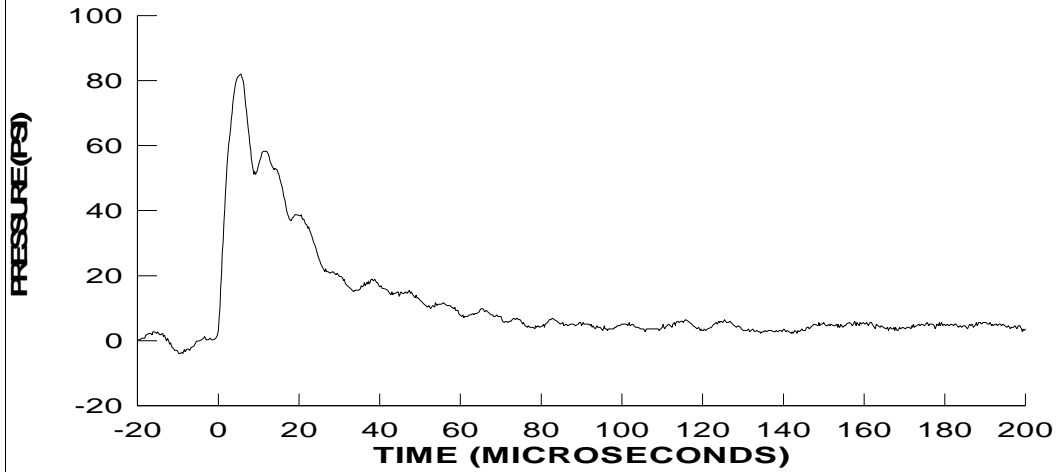


TEST 10 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH

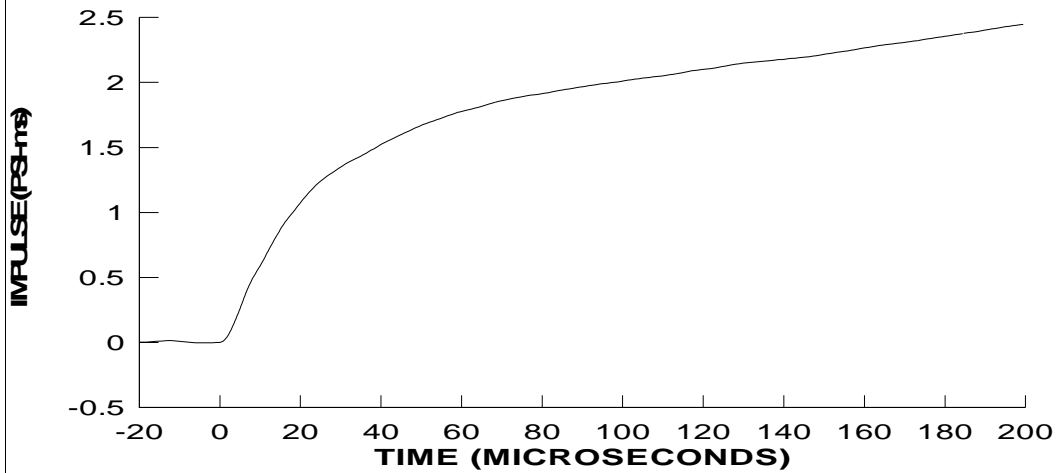




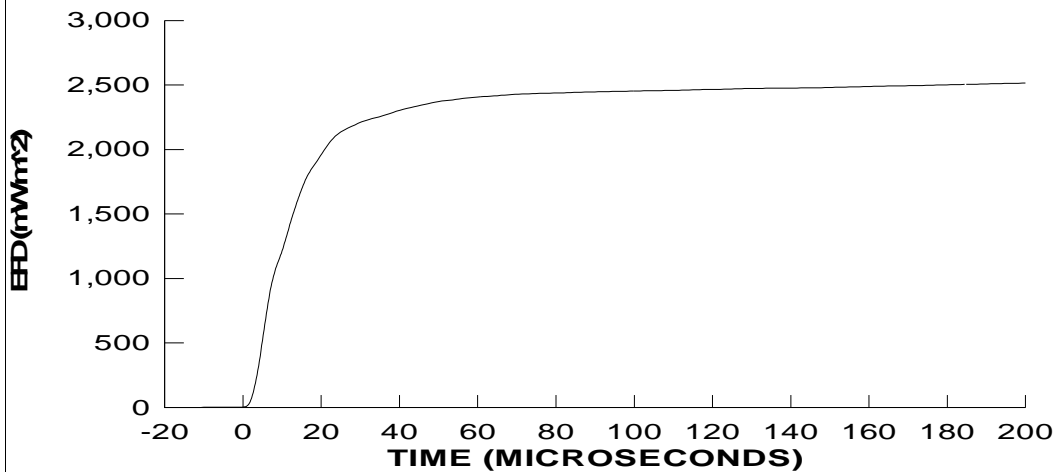
TEST 11 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



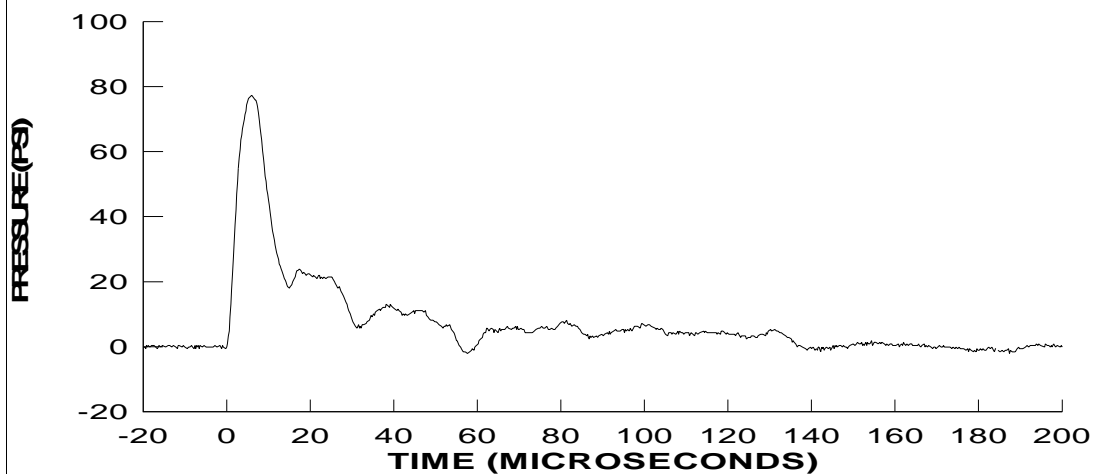
TEST 11 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



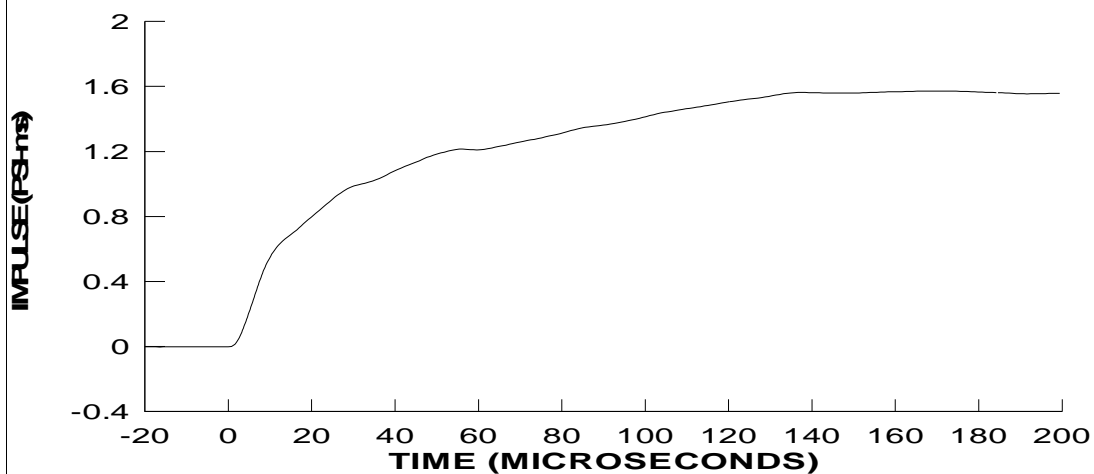
TEST 11 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



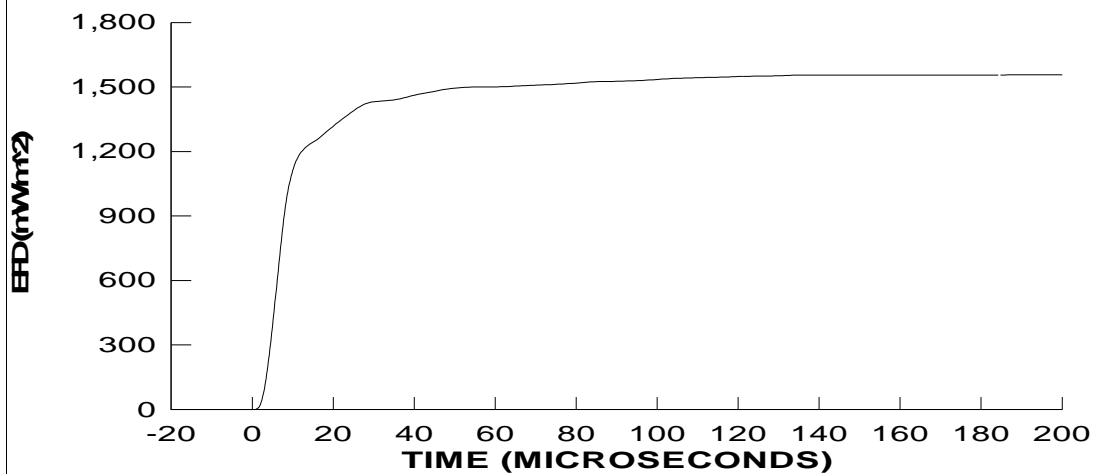
TEST 11 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH



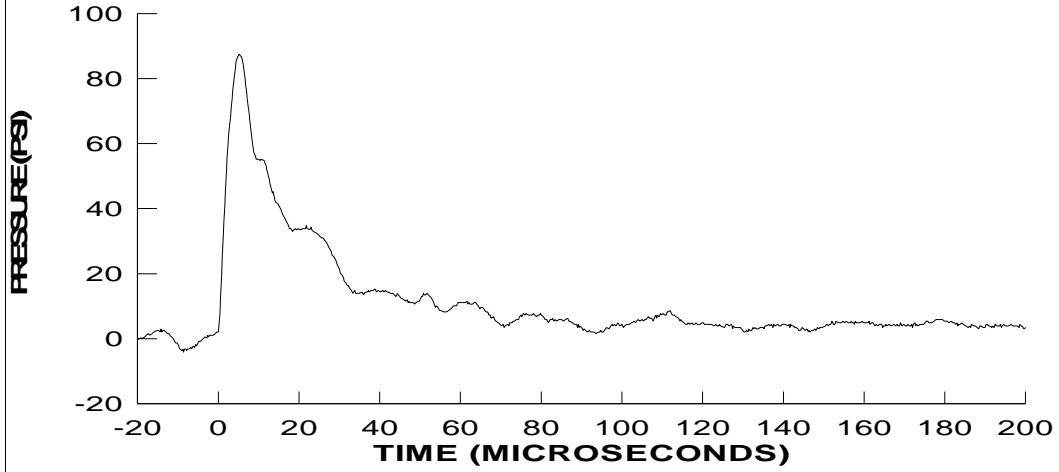
TEST 11 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH



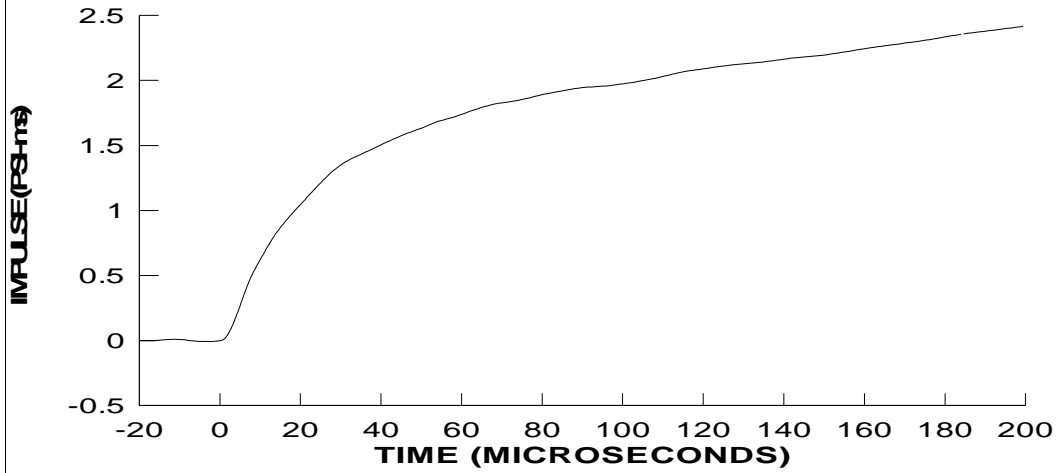
TEST 11 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH



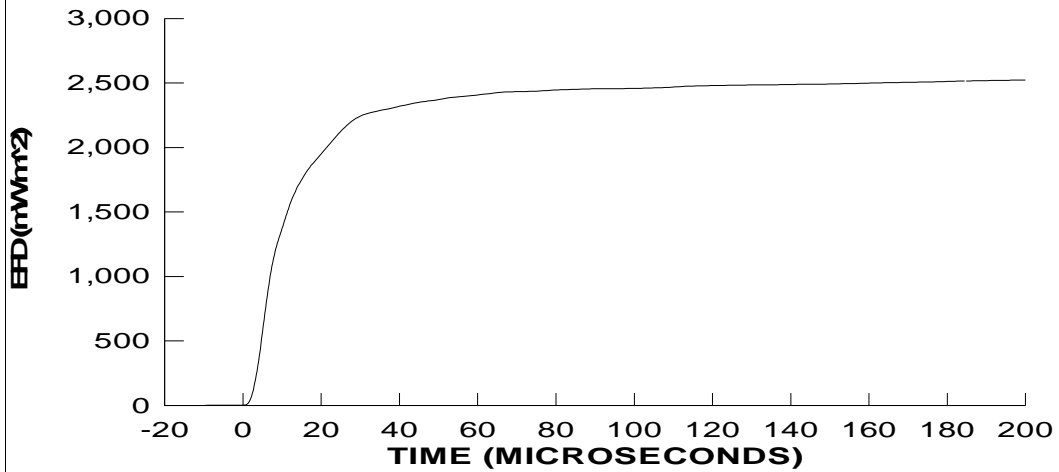
TEST 12 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



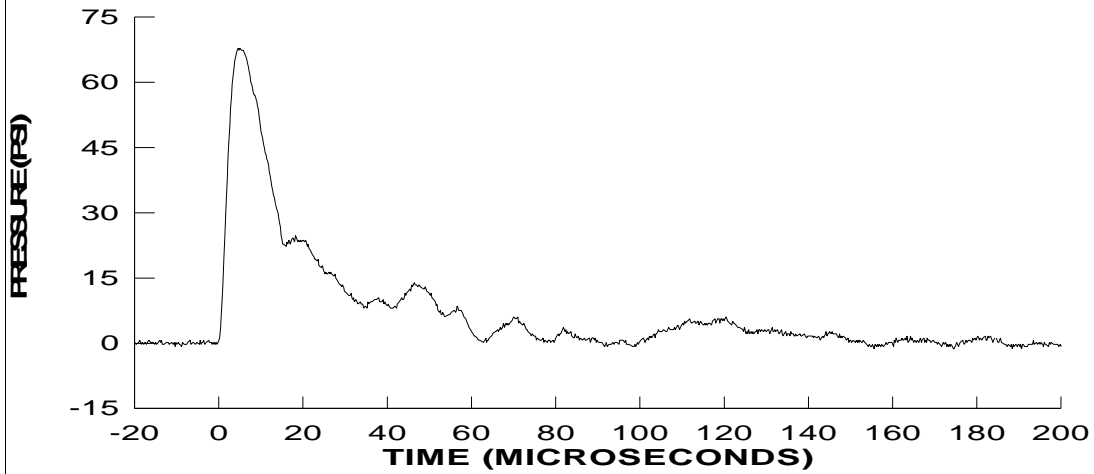
TEST 12 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



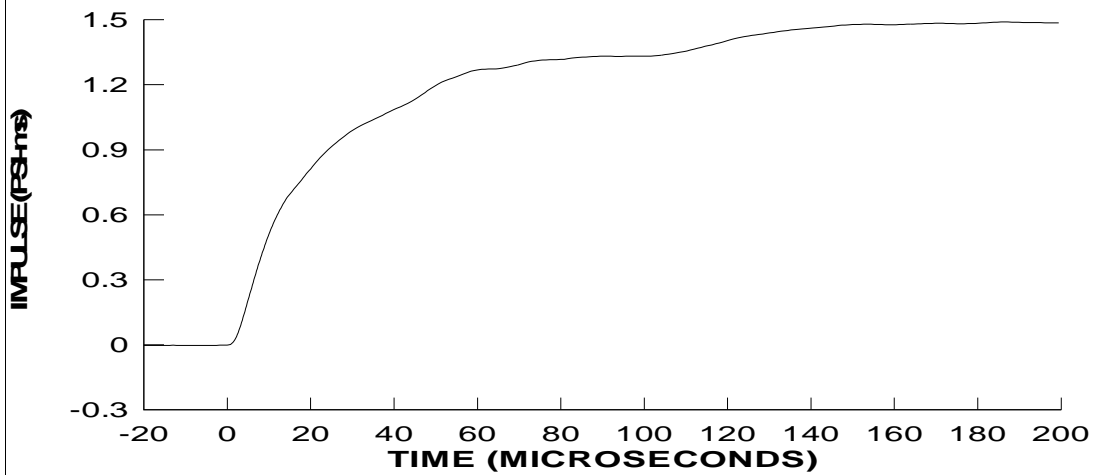
TEST 12 TRANSDUCER 1
3.6 METERS OUTSIDE BAG NO FISH



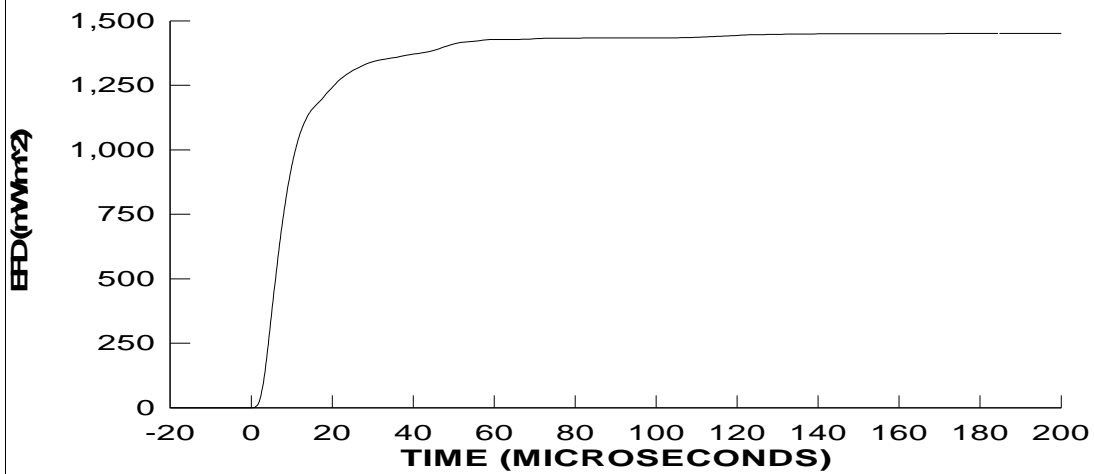
TEST 12 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH

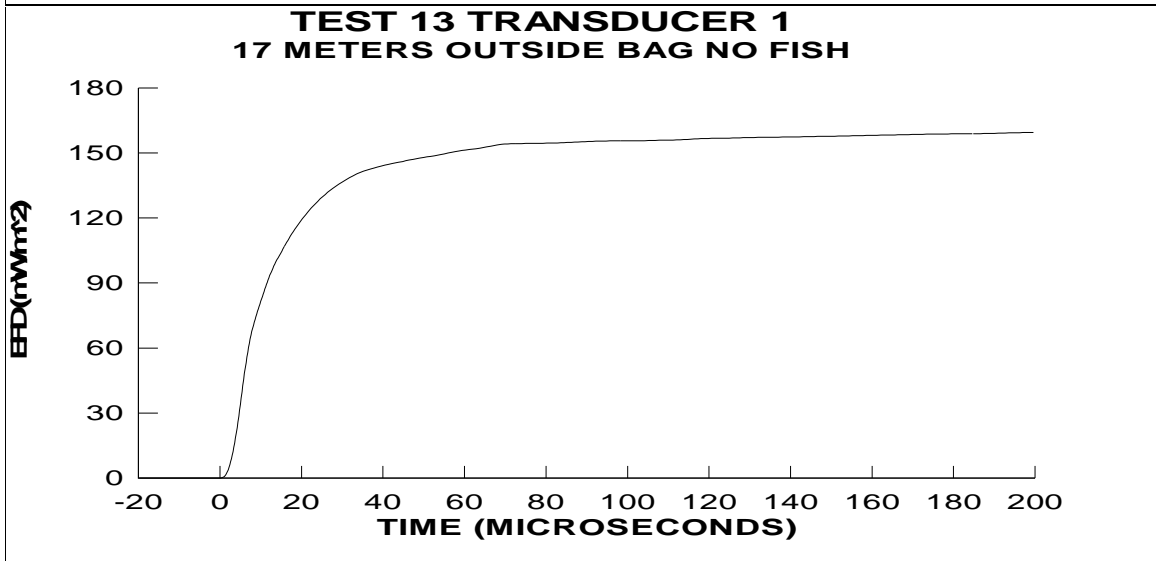
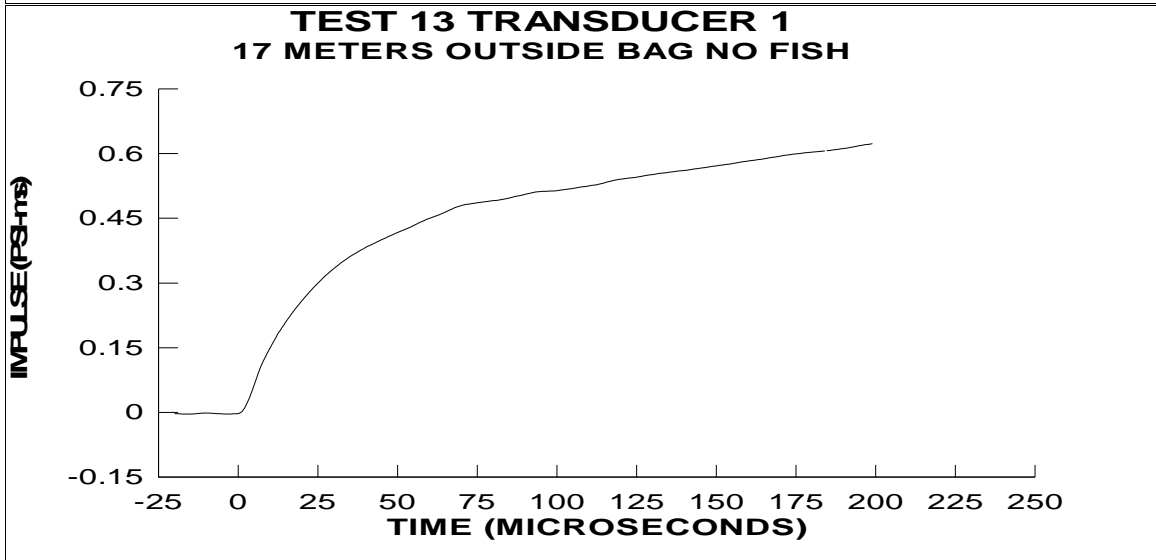
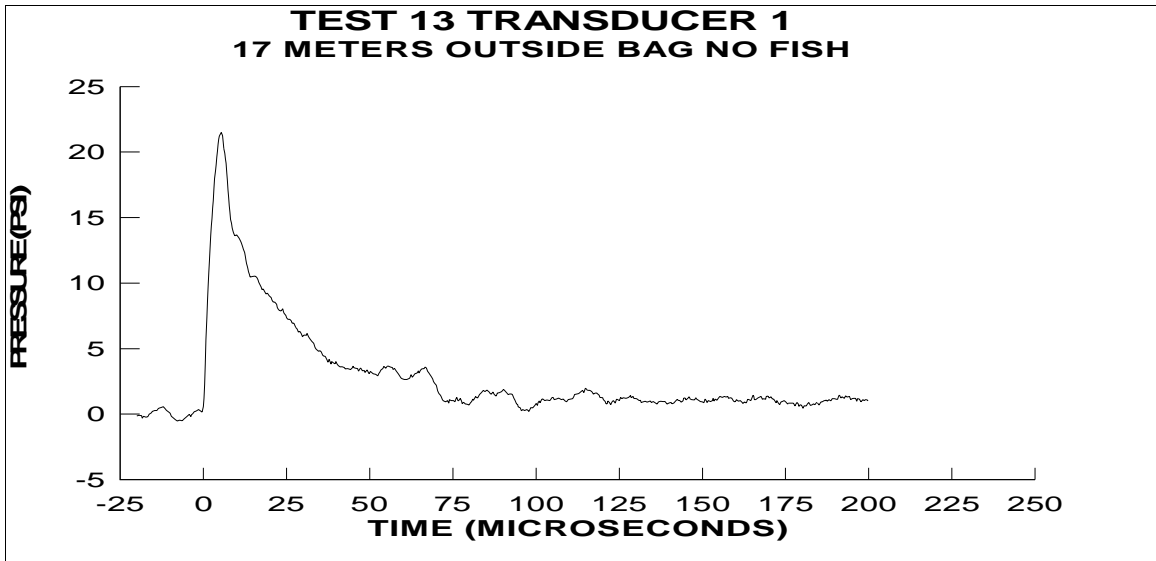


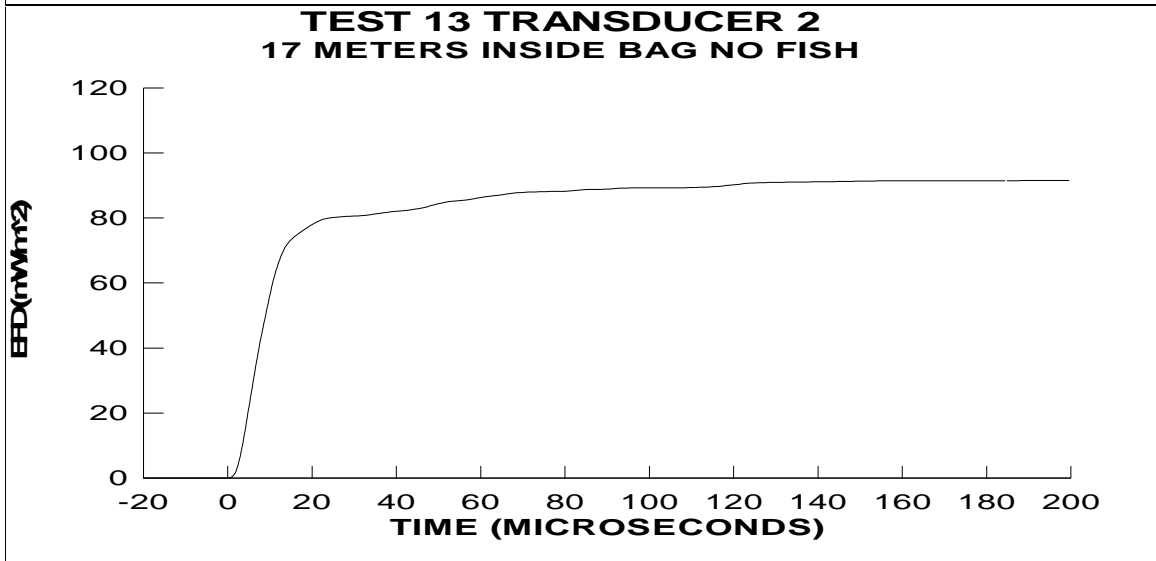
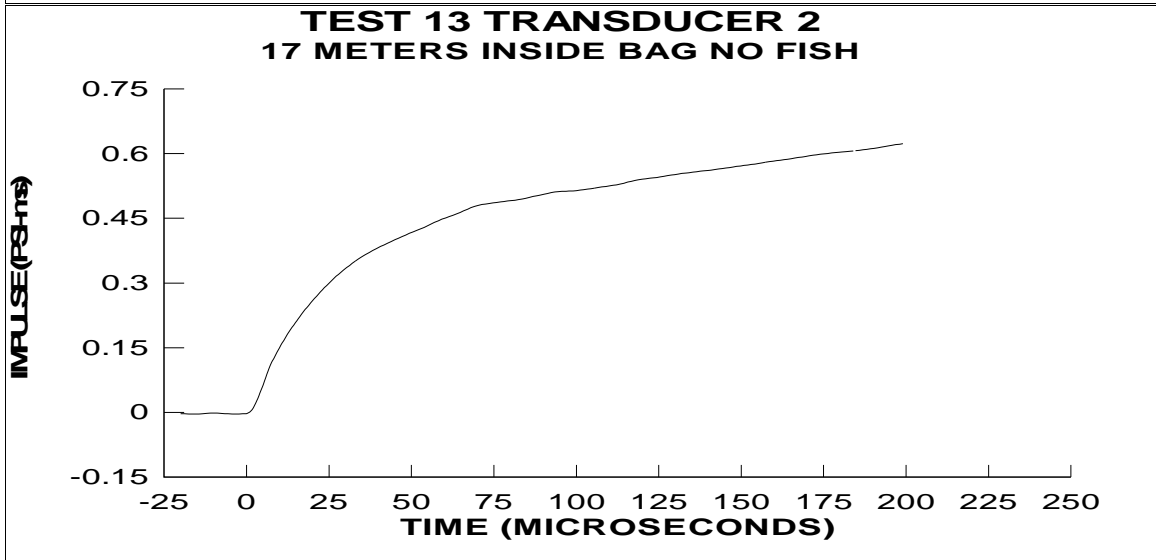
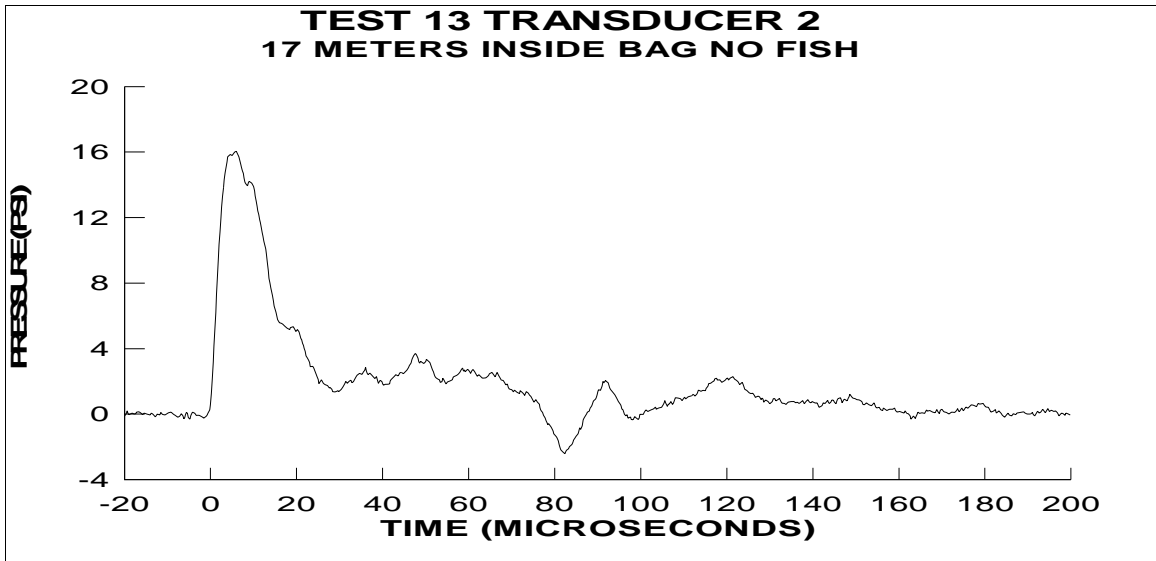
TEST 12 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH



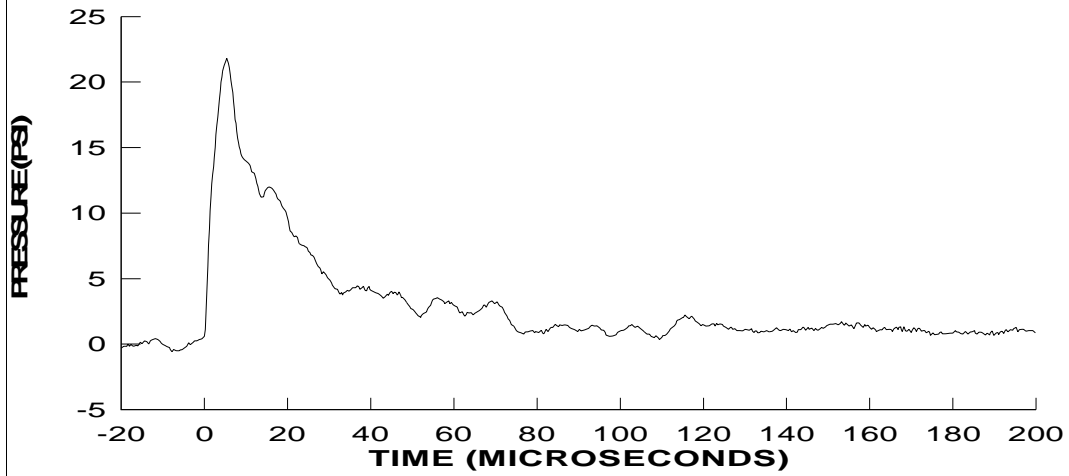
TEST 12 TRANSDUCER 2
3.6 METERS INSIDE BAG NO FISH



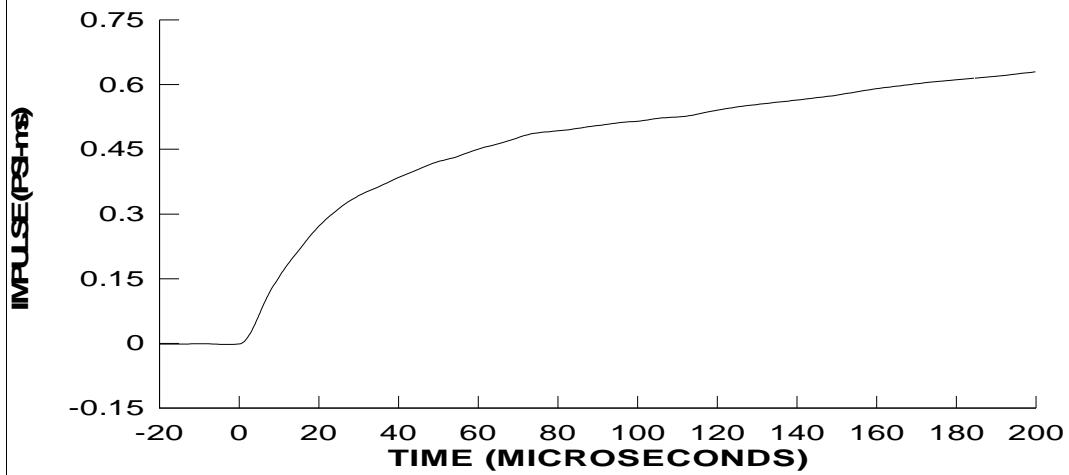




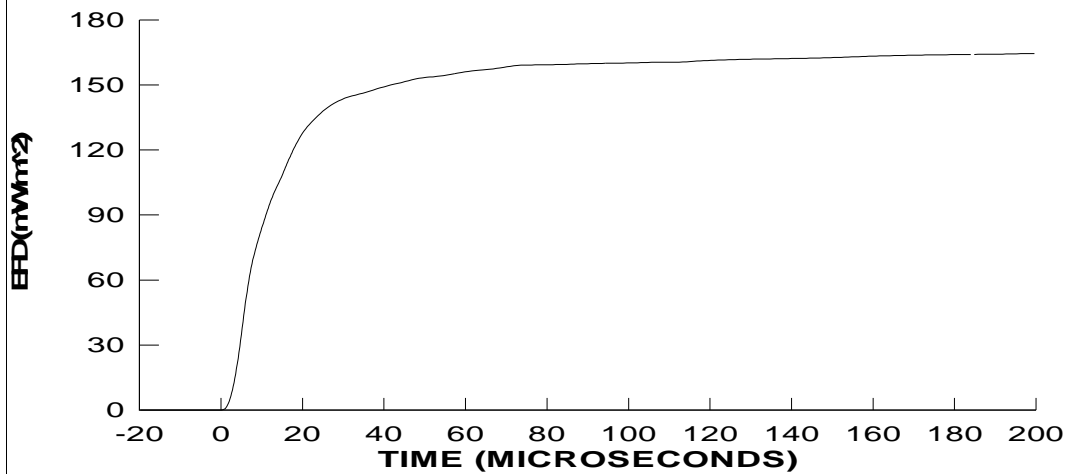
TEST 14 TRANSDUCER 1
17 METERS OUTSIDE BAG NO FISH

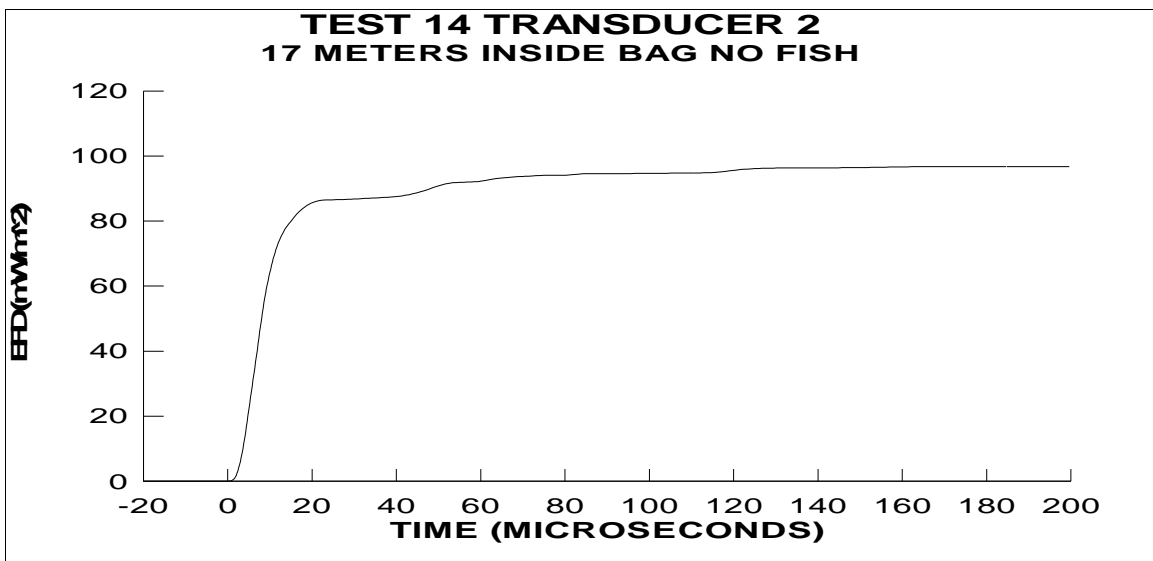
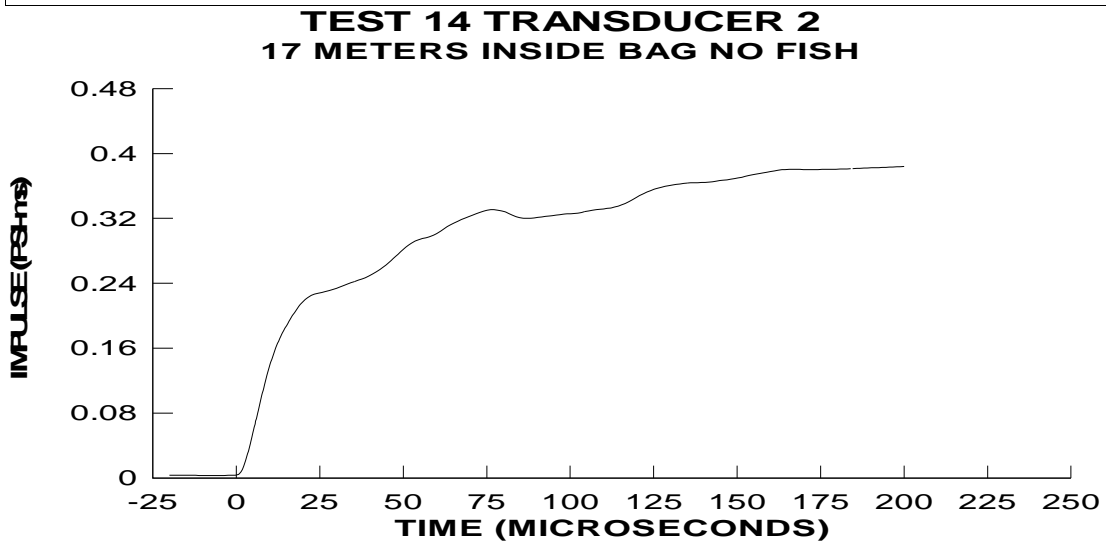
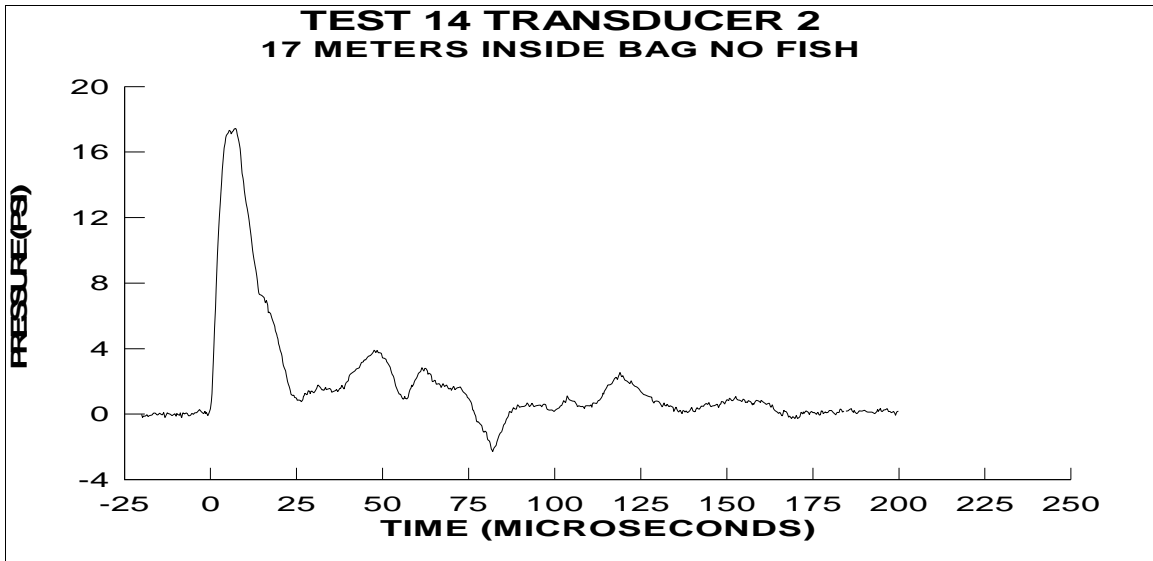


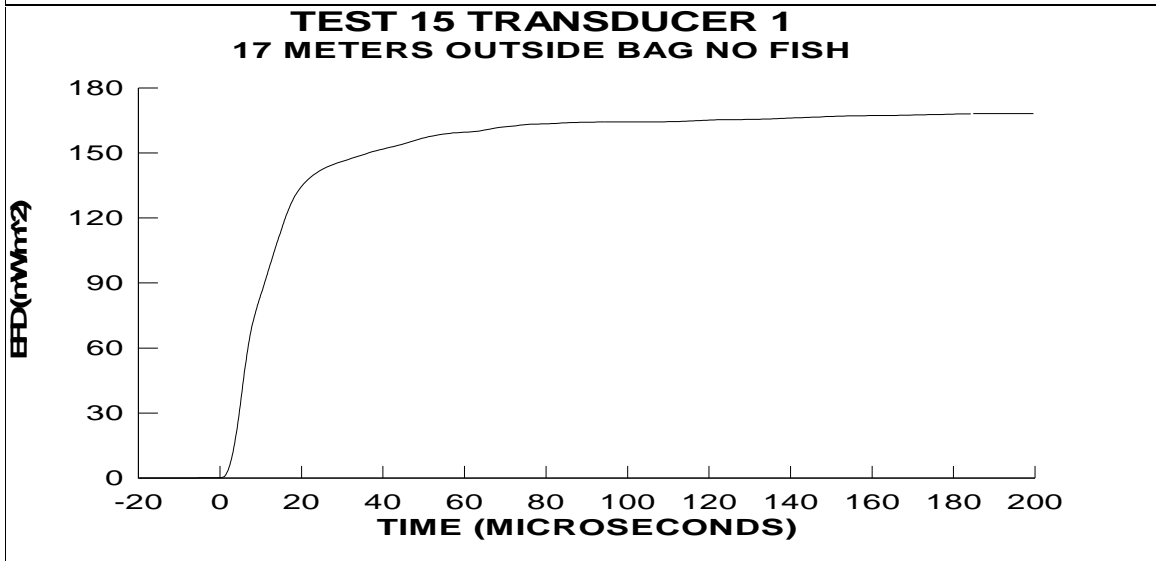
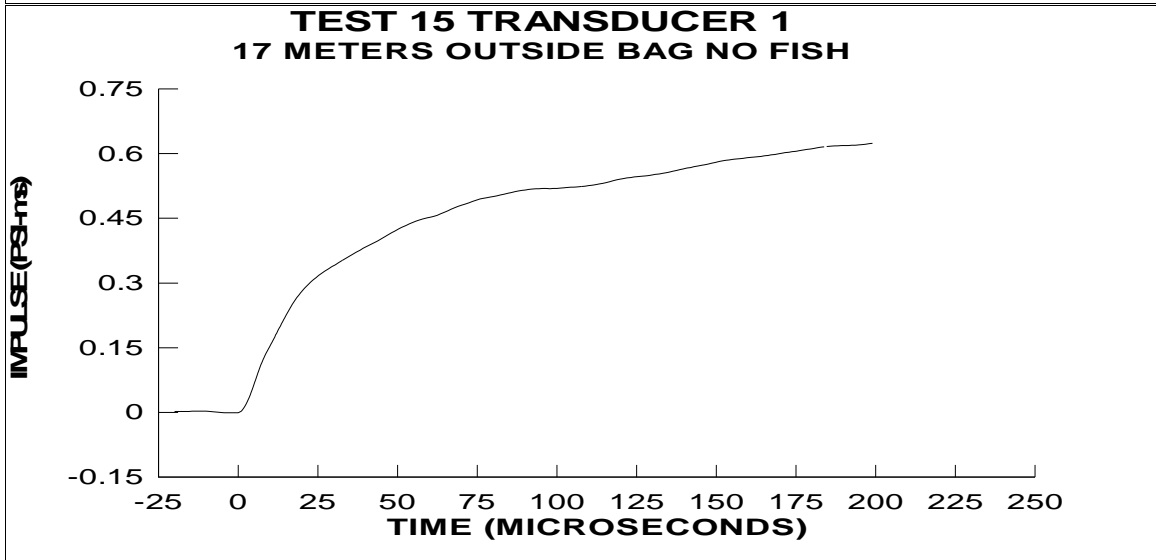
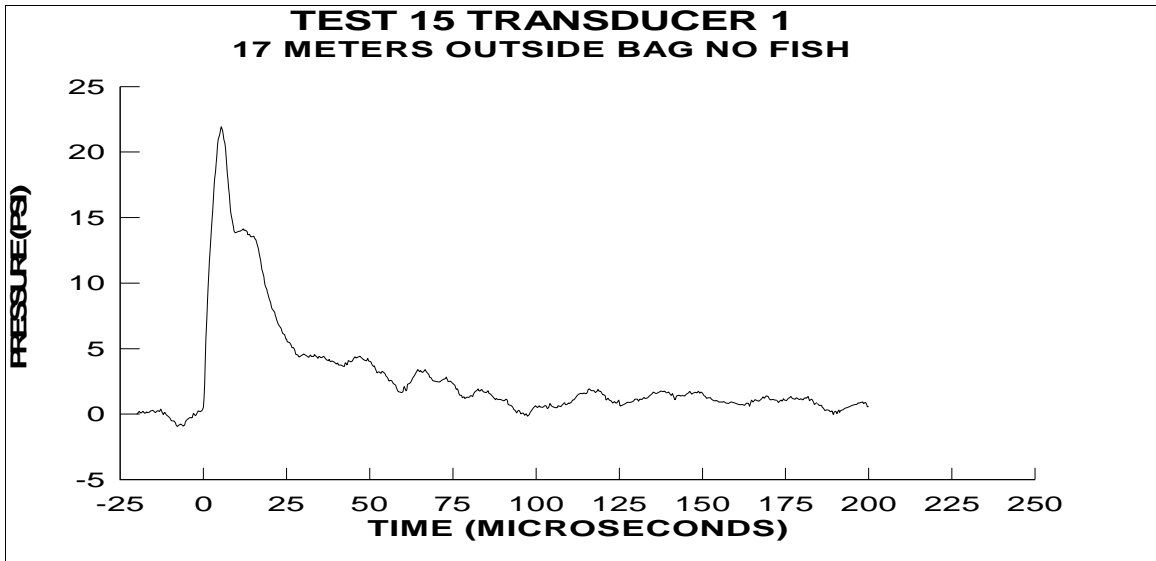
TEST 14 TRANSDUCER 1
17 METERS OUTSIDE BAG NO FISH

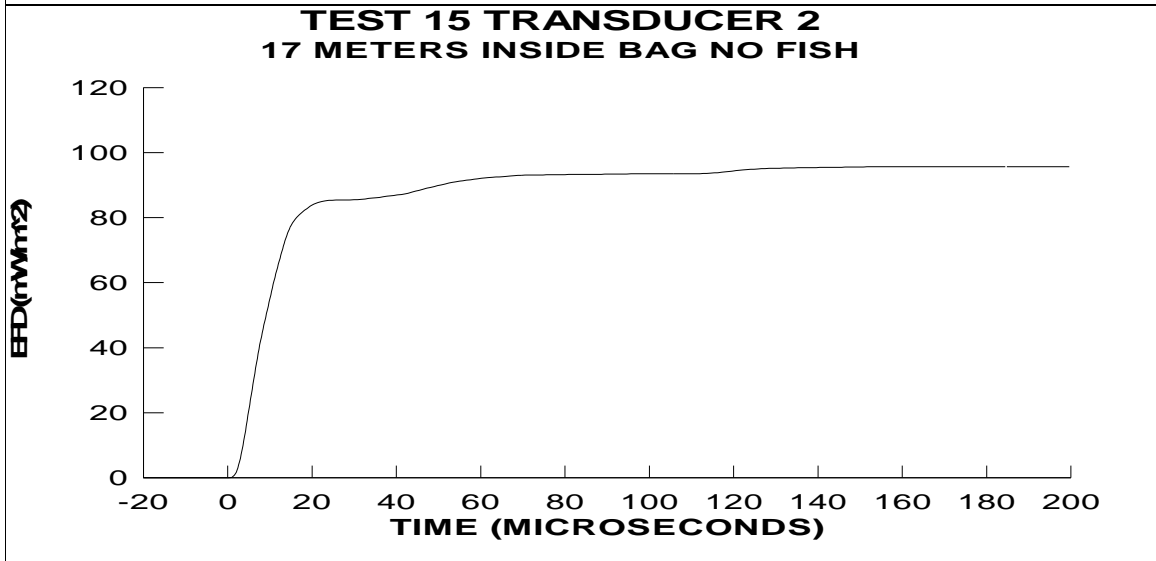
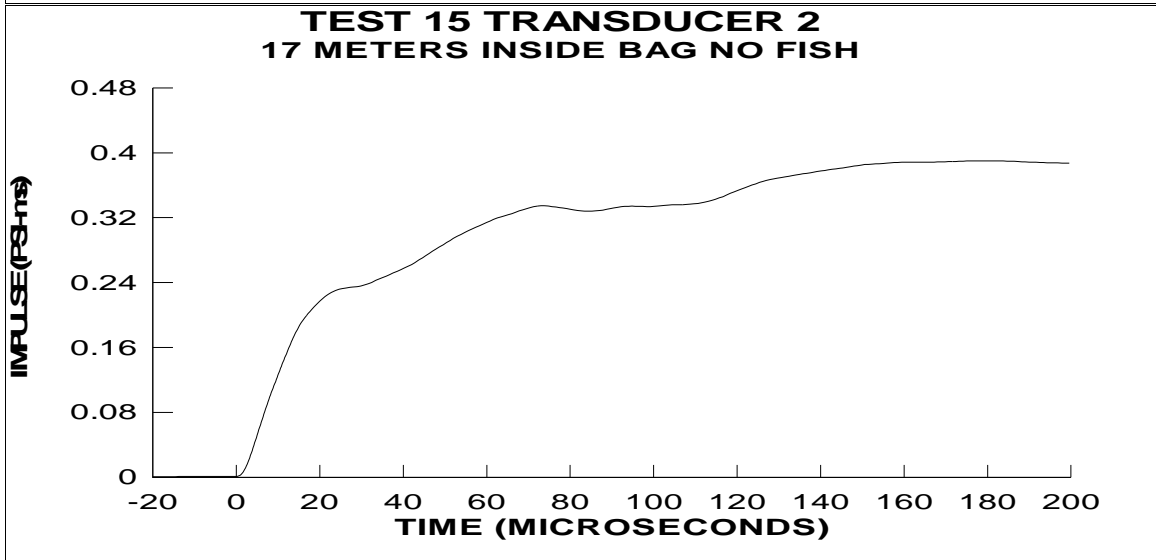
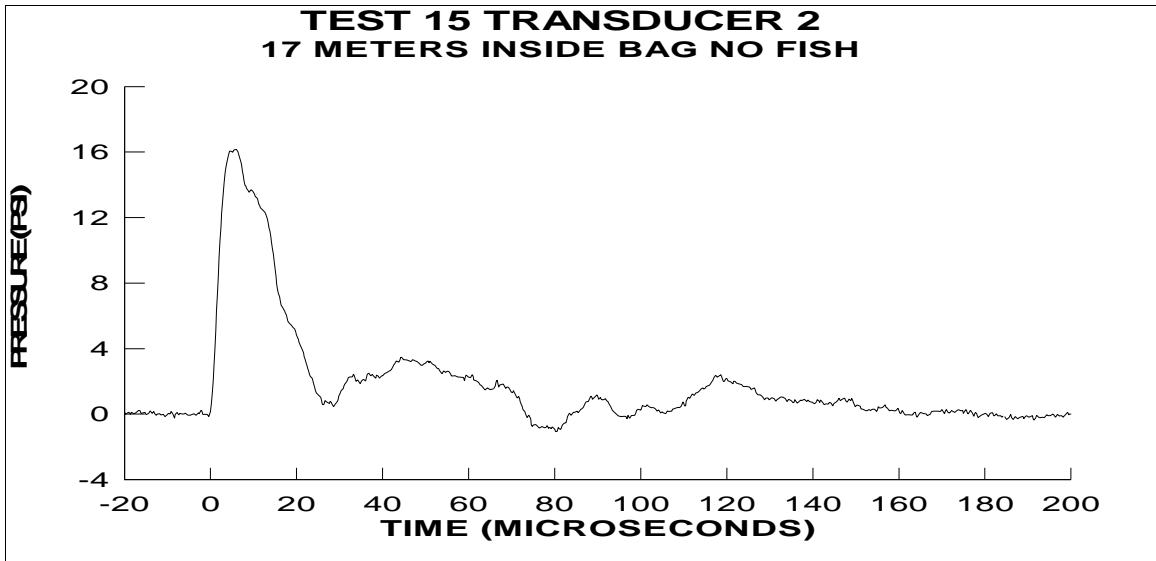


TEST 14 TRANSDUCER 1
17 METERS OUTSIDE BAG NO FISH

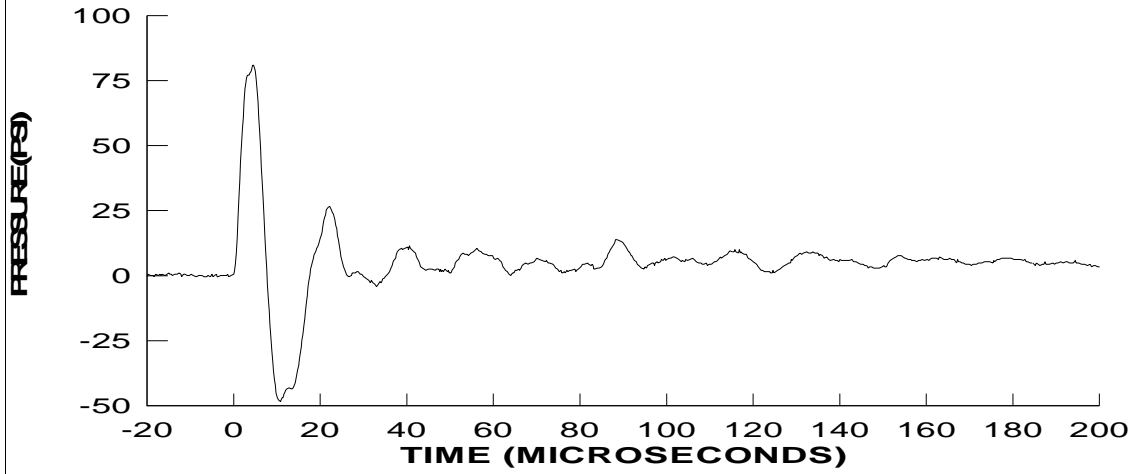




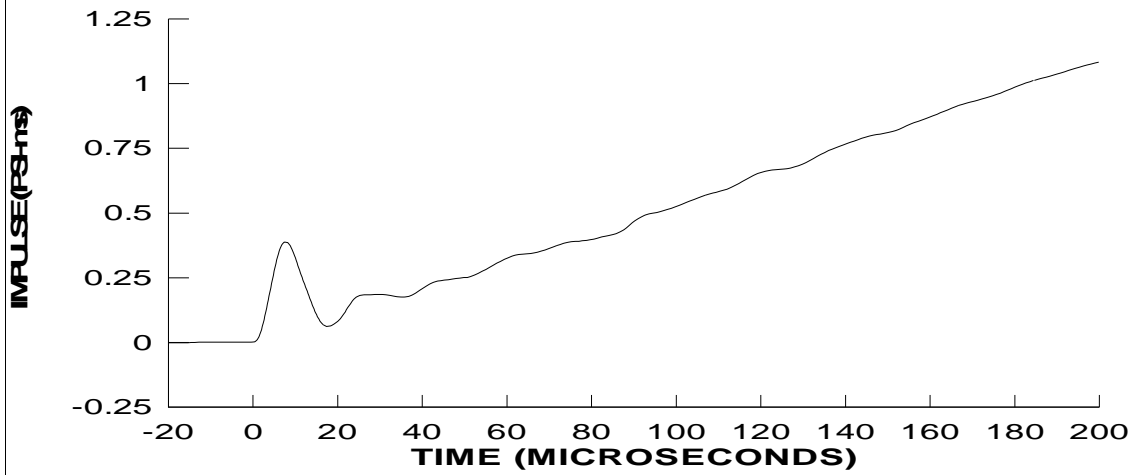




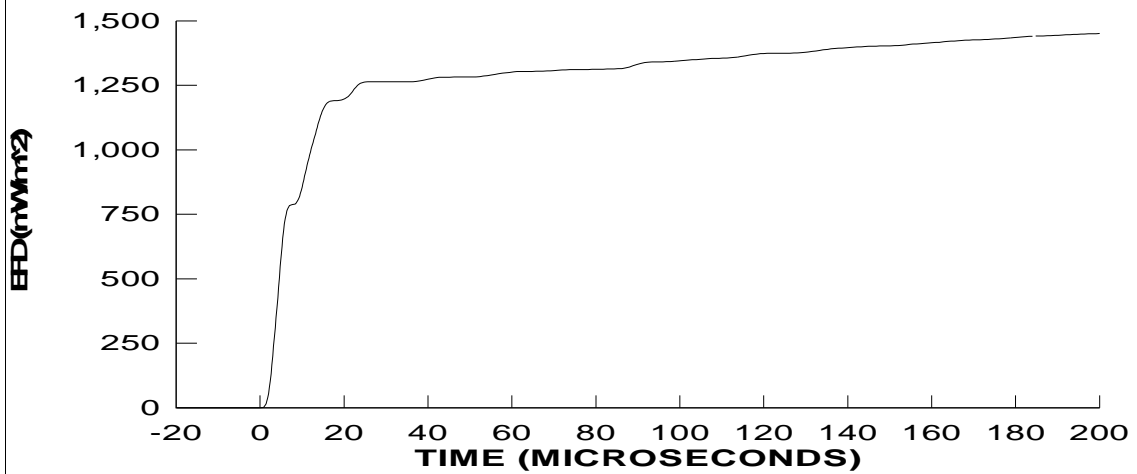
TEST 16 TRANSDUCER 1
3.6 METERS PORT WITH FISH



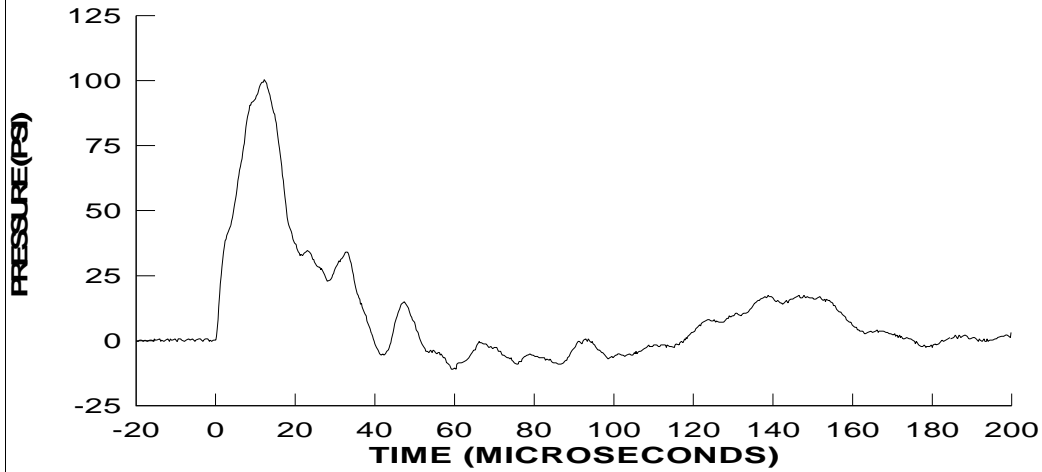
TEST 16 TRANSDUCER 1
3.6 METERS PORT WITH FISH



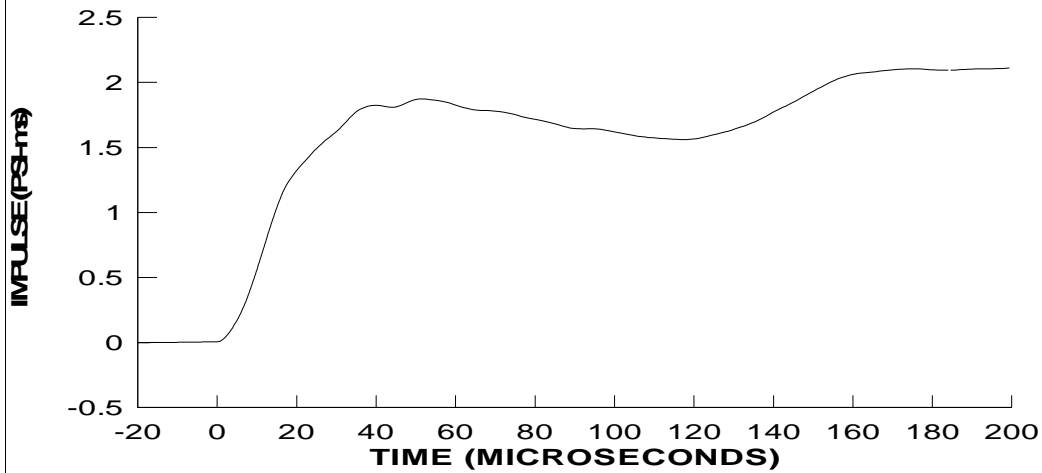
TEST 16 TRANSDUCER 1
3.6 METERS PORT WITH FISH



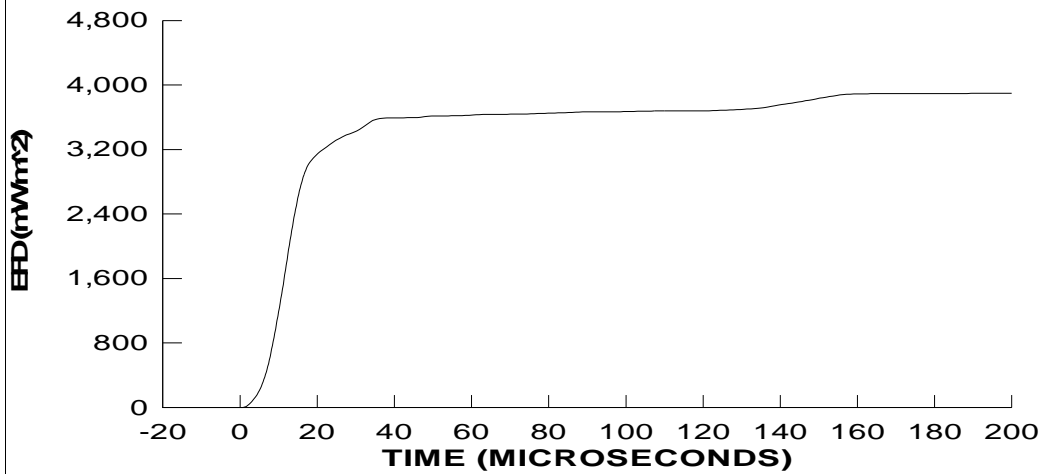
TEST 16 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

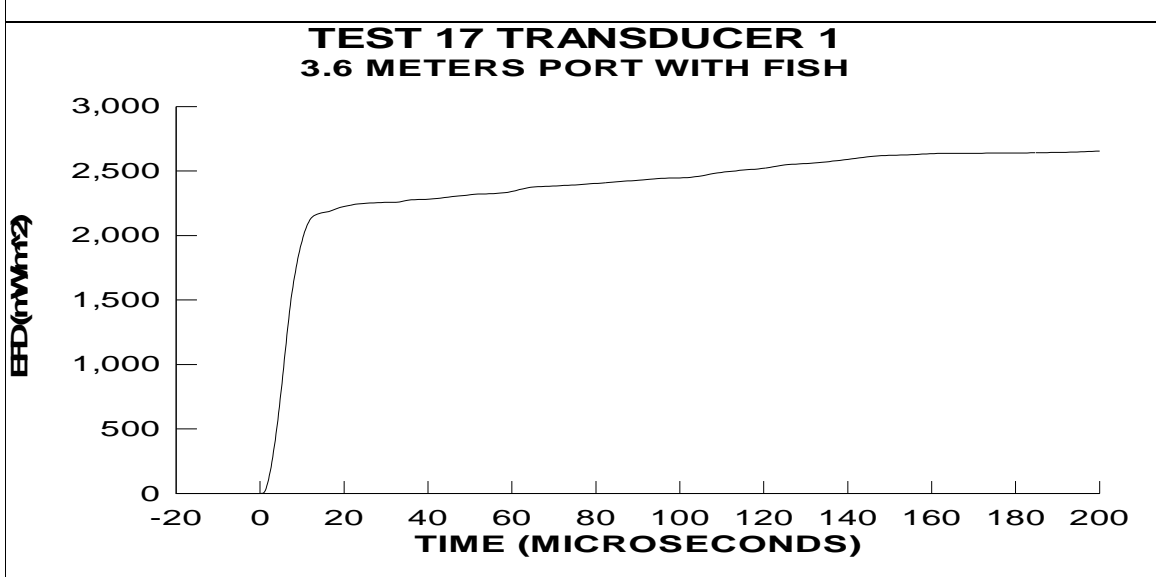
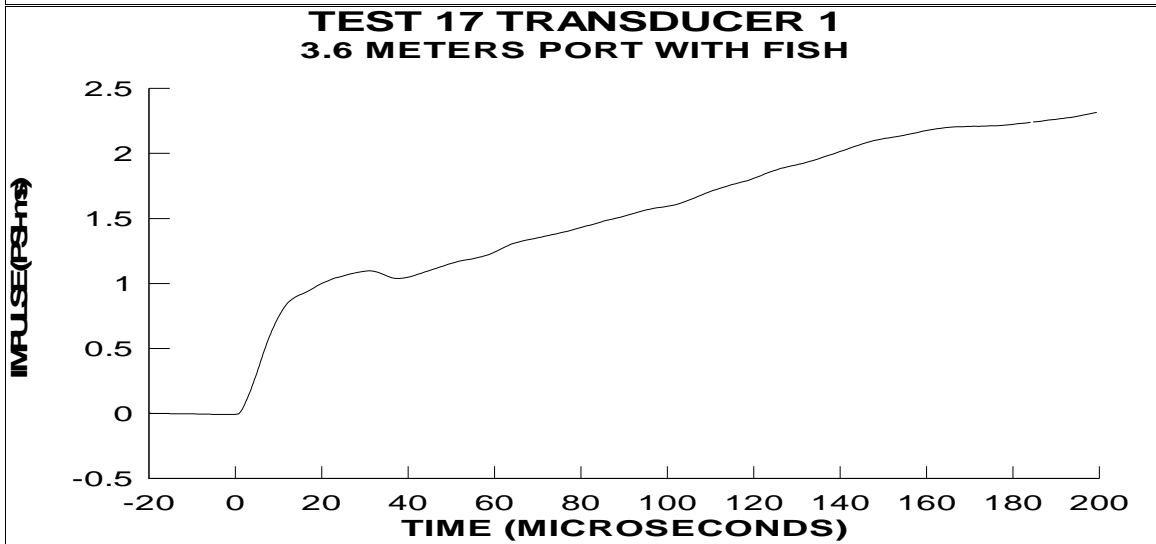
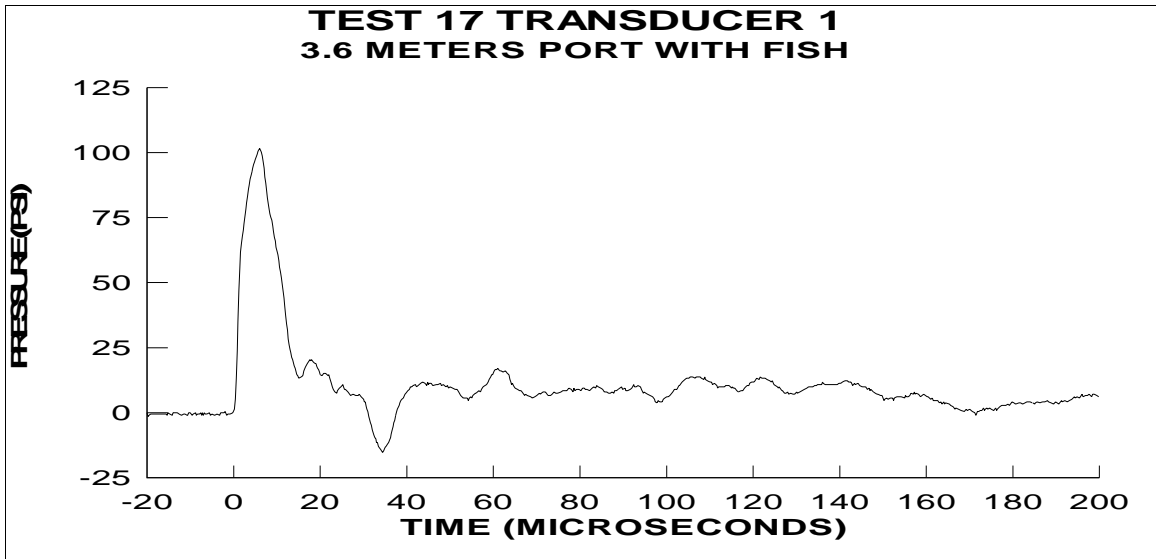


TEST 16 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

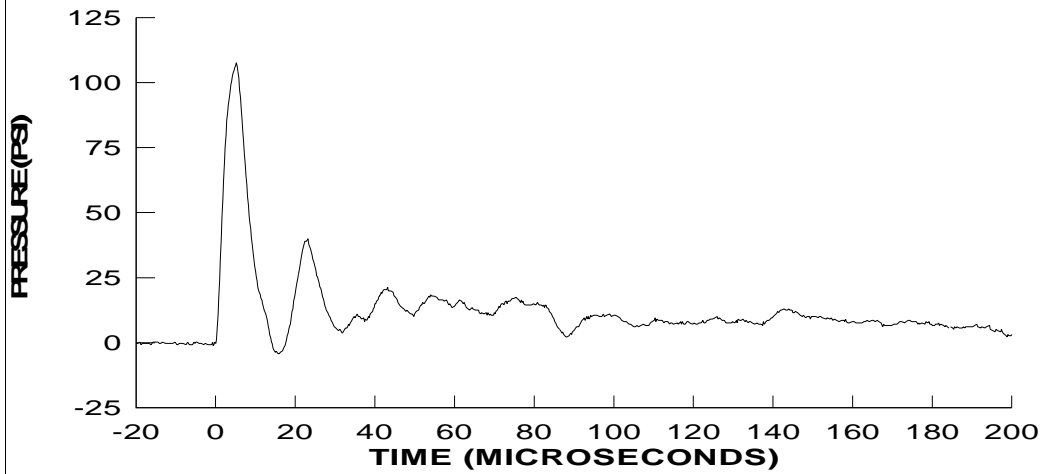


TEST 16 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

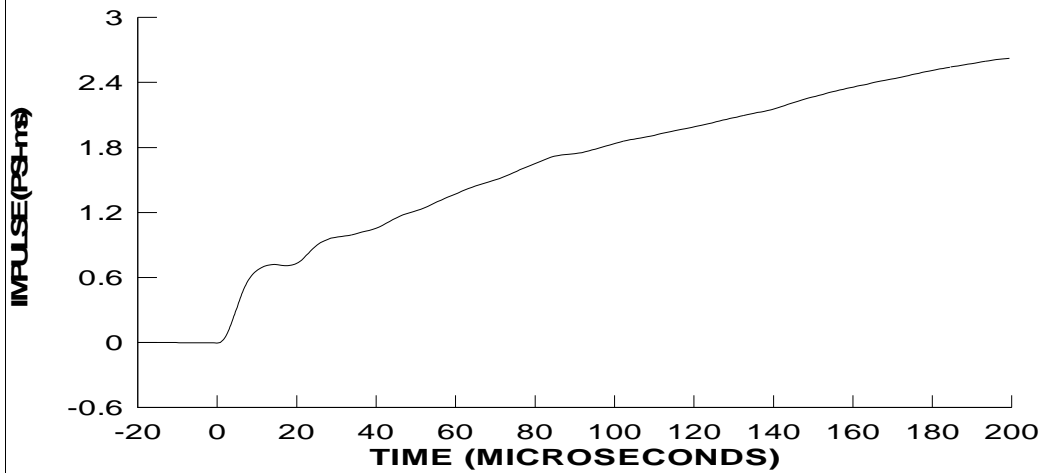




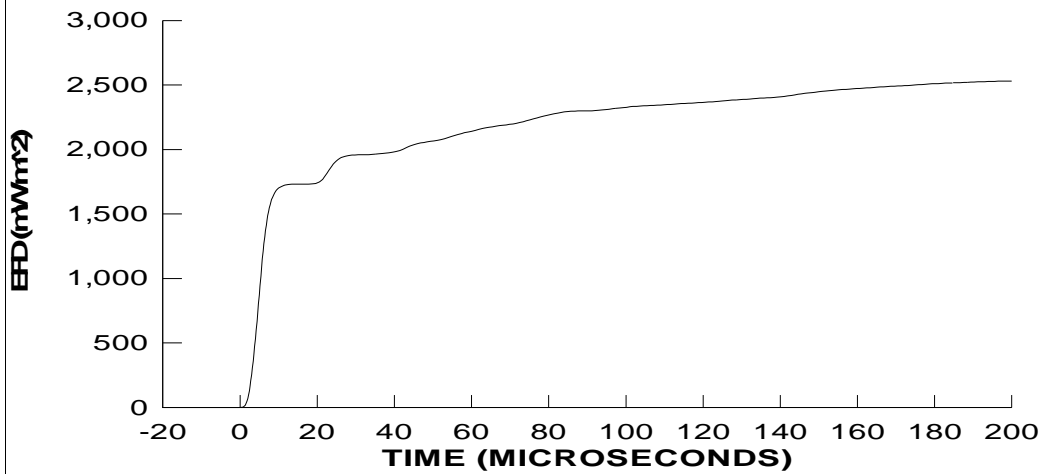
TEST 17 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

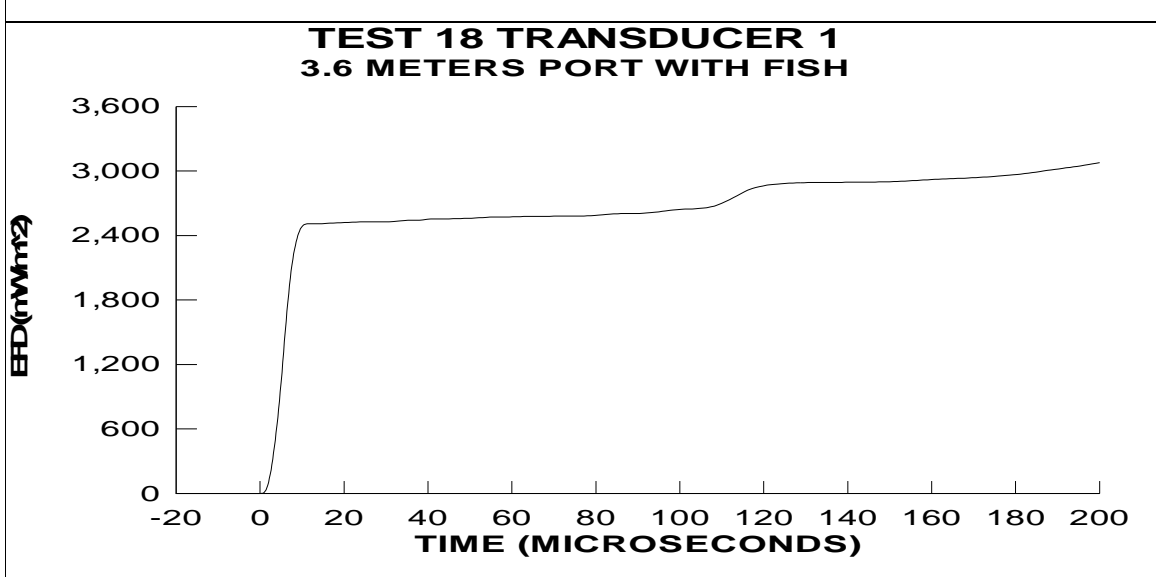
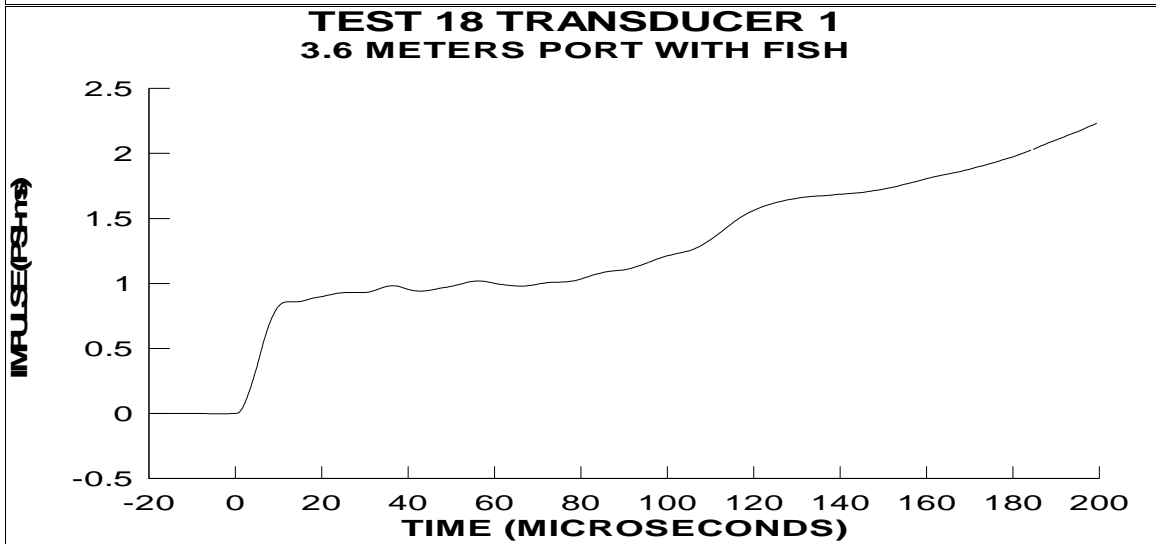
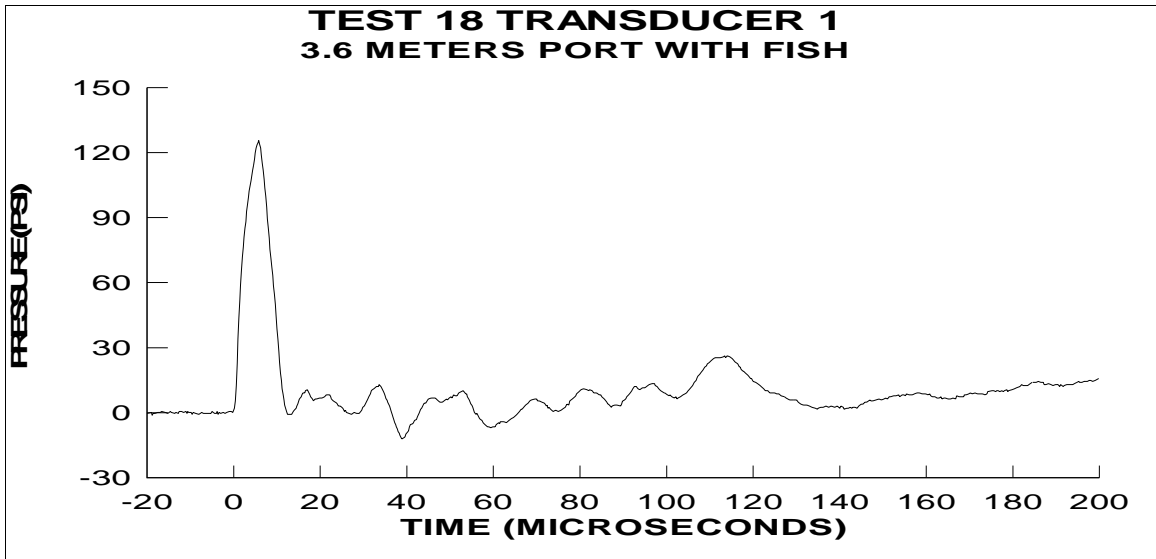


TEST 17 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

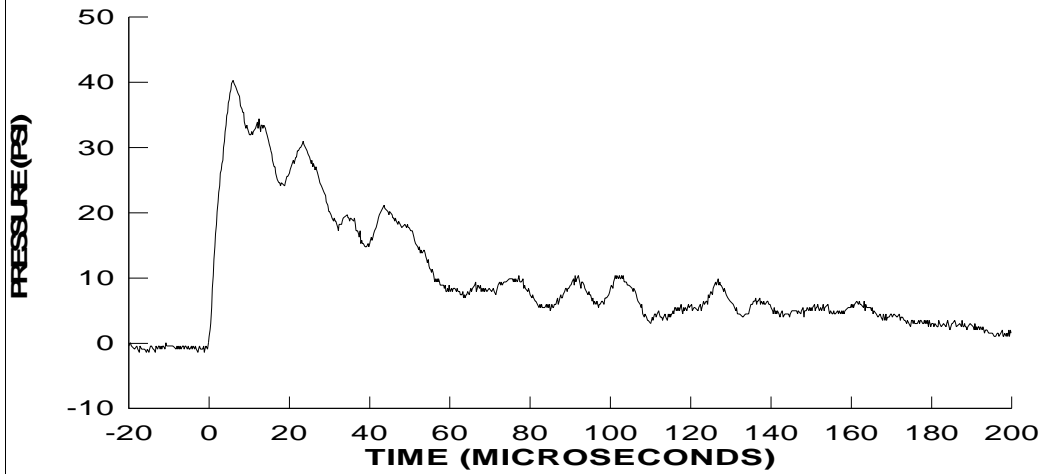


TEST 17 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

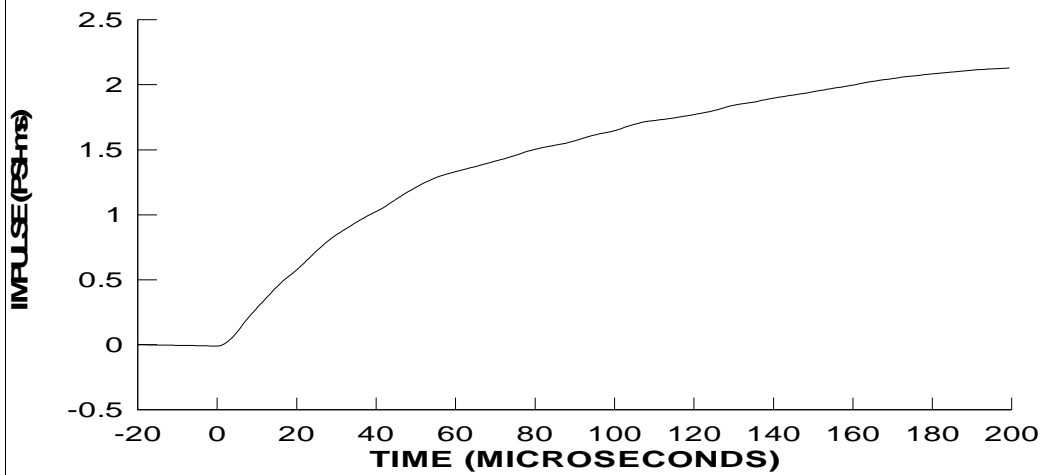




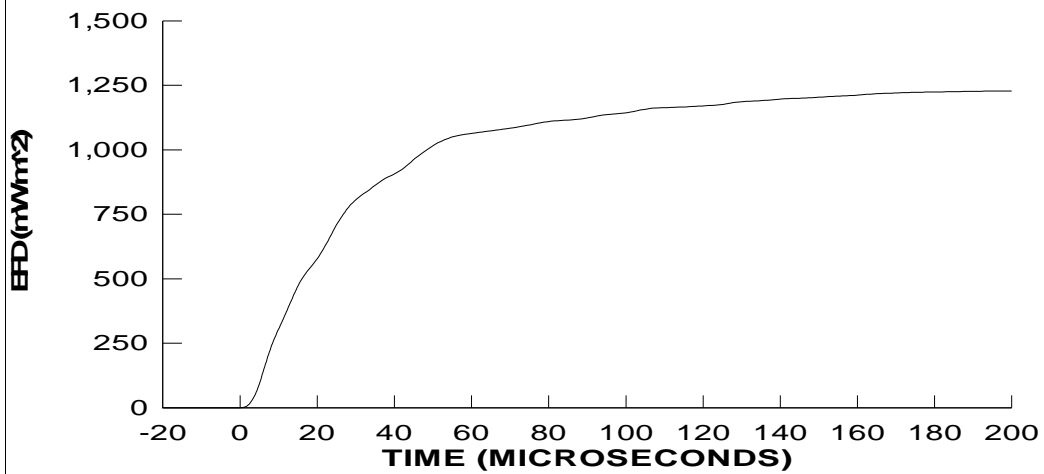
TEST 18 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

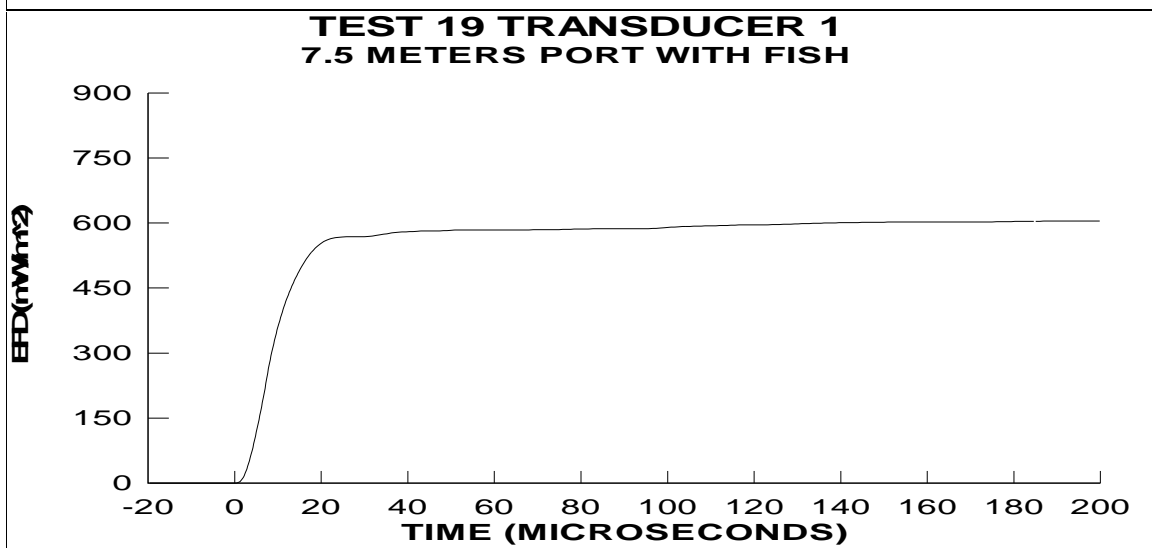
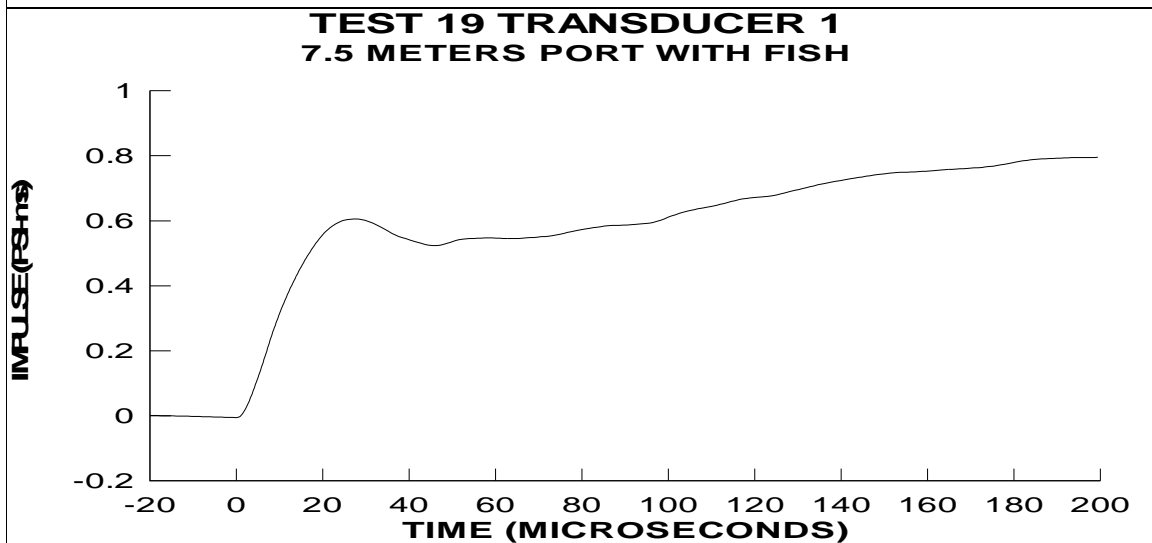
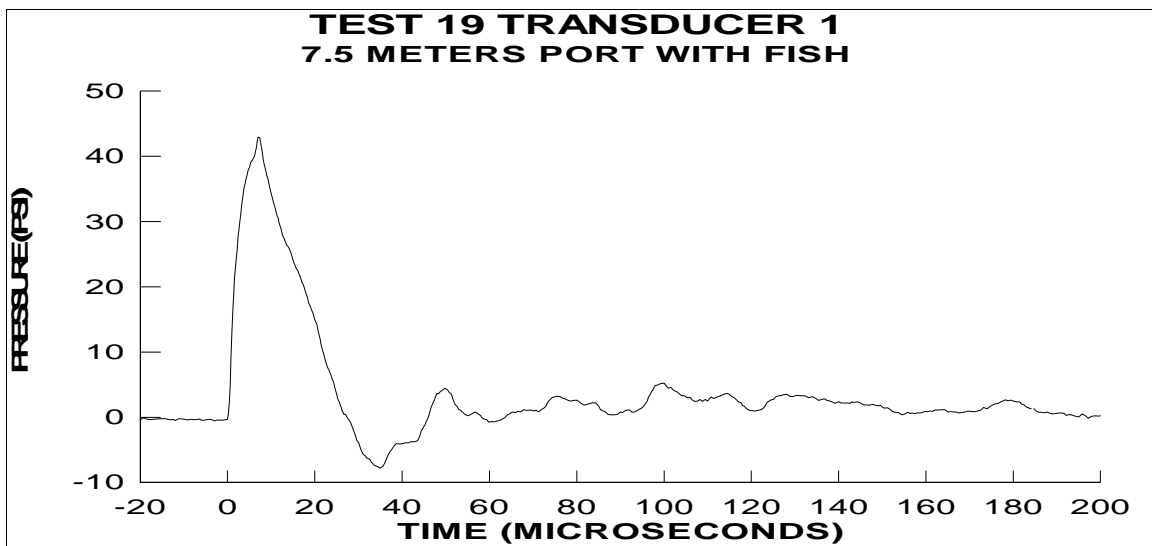


TEST 18 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

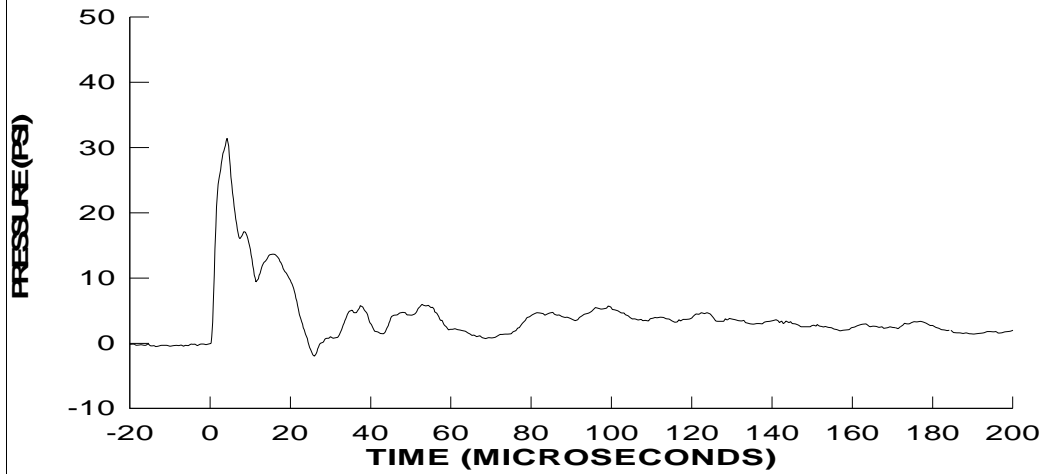


TEST 18 TRANSDUCER 2
3.6 METERS STARBOARD WITH FISH

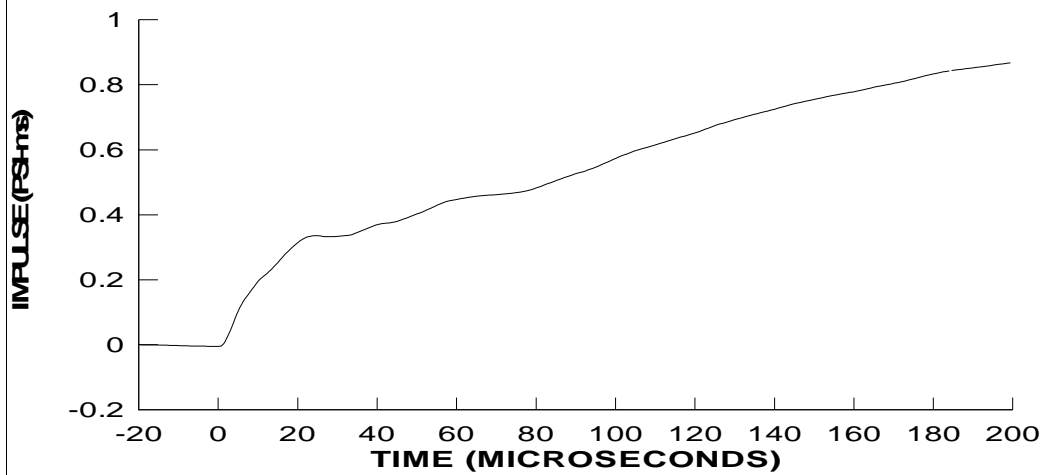




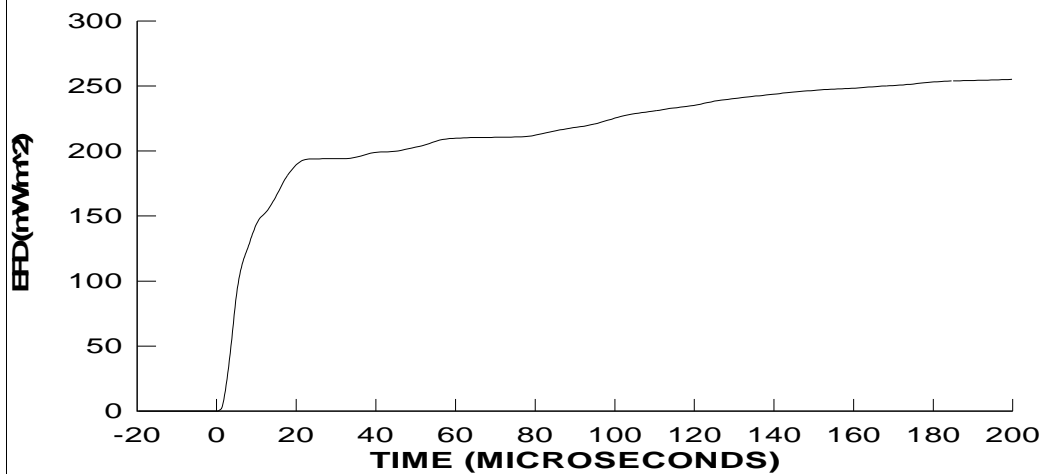
TEST 19 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



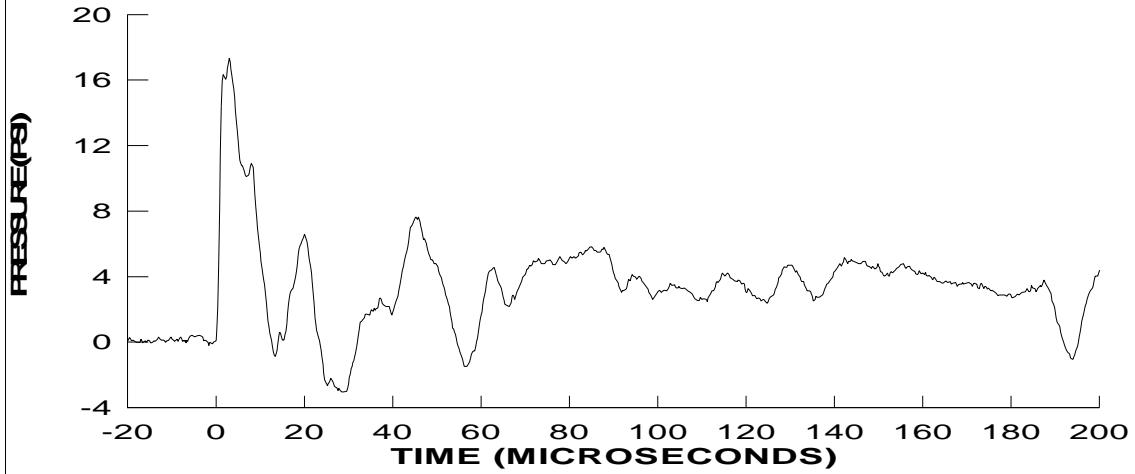
TEST 19 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



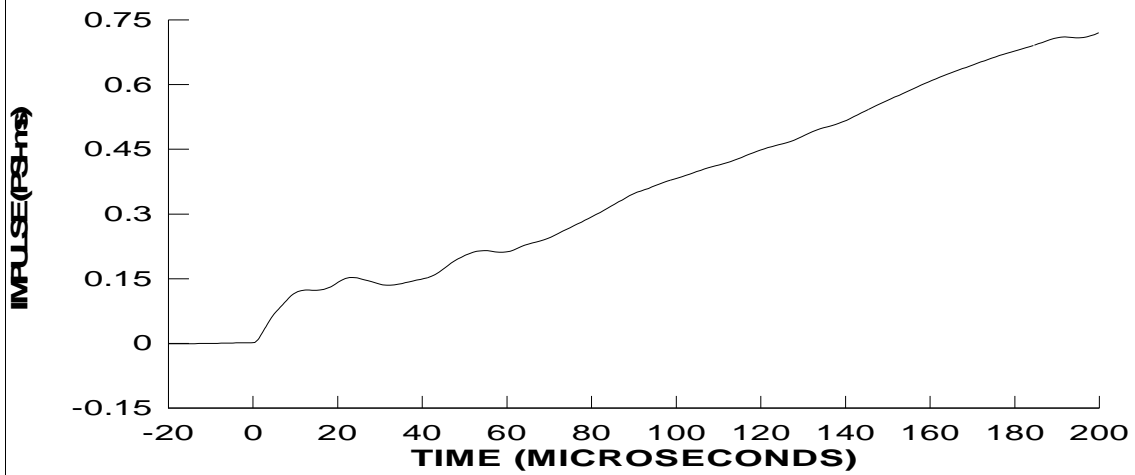
TEST 19 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



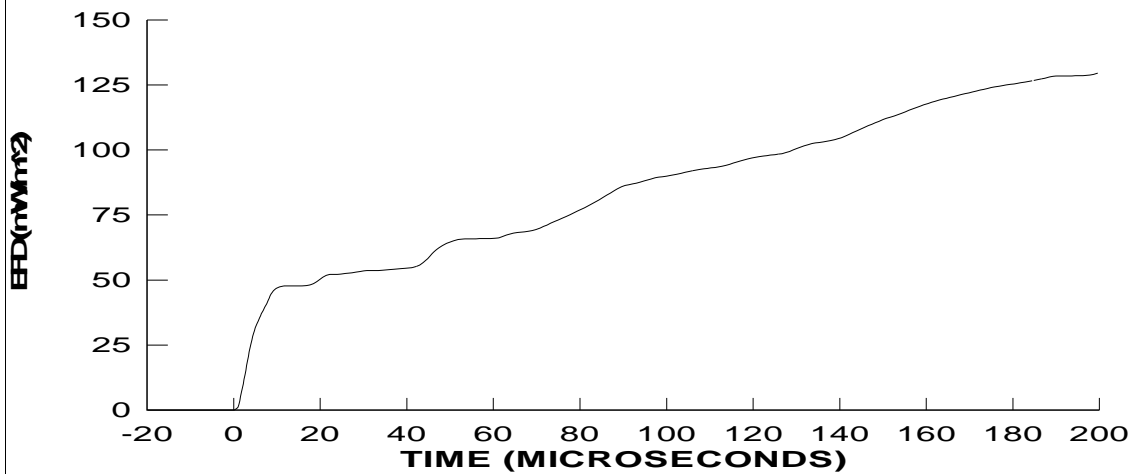
TEST 20 TRANSDUCER 1
7.5 METERS PORT WITH FISH



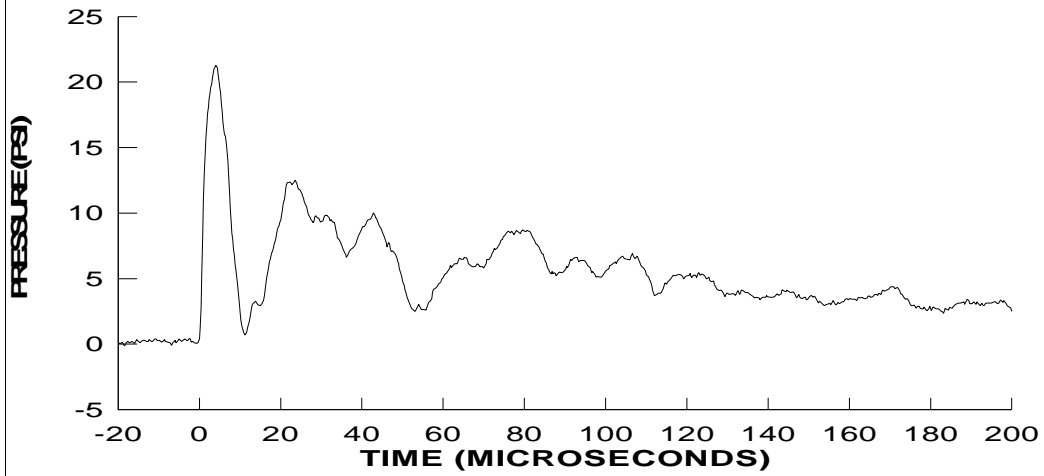
TEST 20 TRANSDUCER 1
7.5 METERS PORT WITH FISH



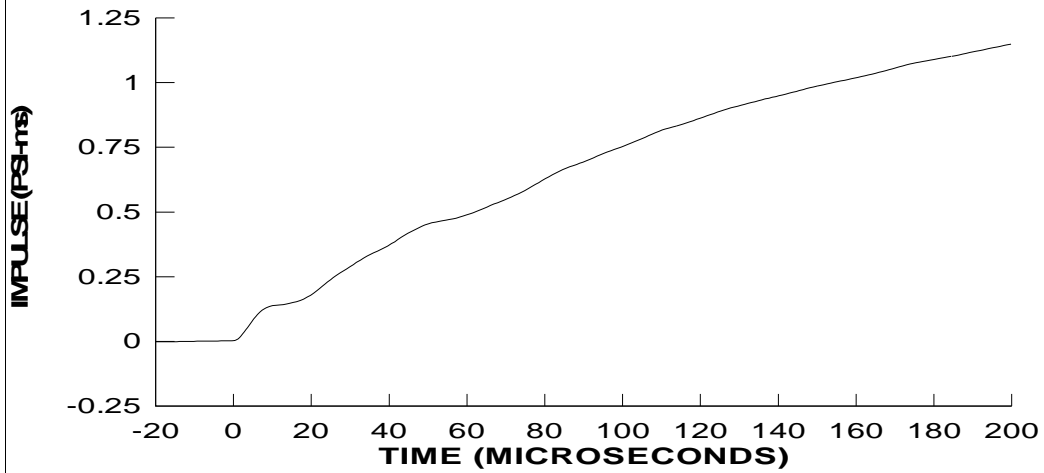
TEST 20 TRANSDUCER 1
7.5 METERS PORT WITH FISH



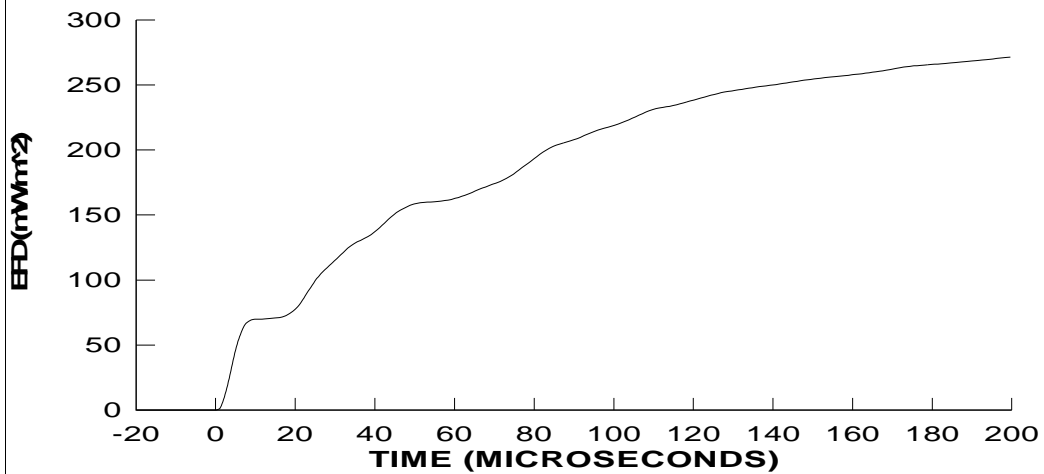
TEST 20 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH

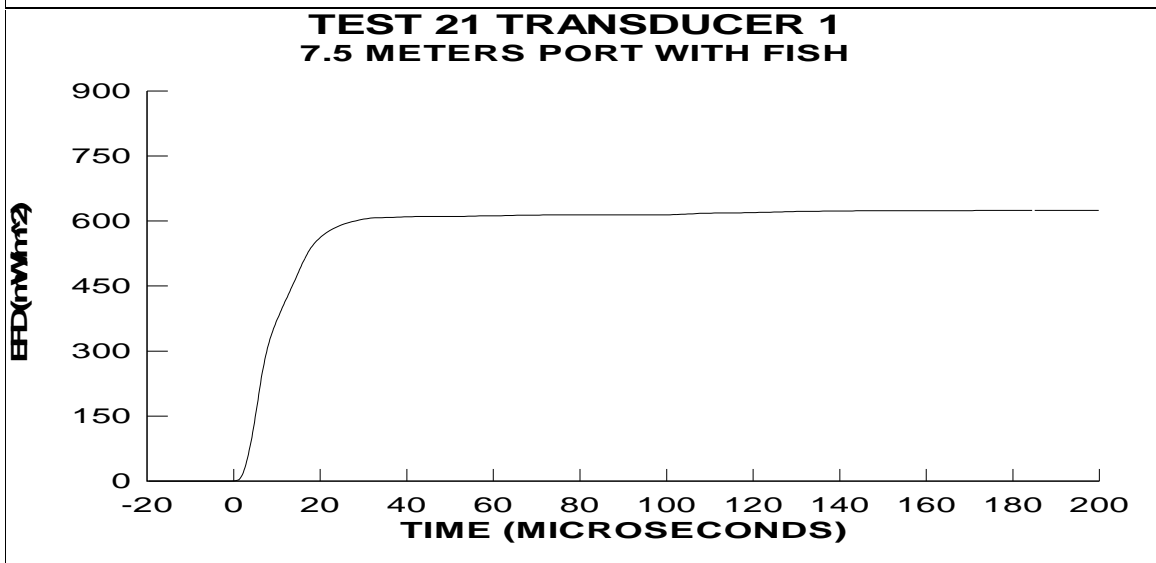
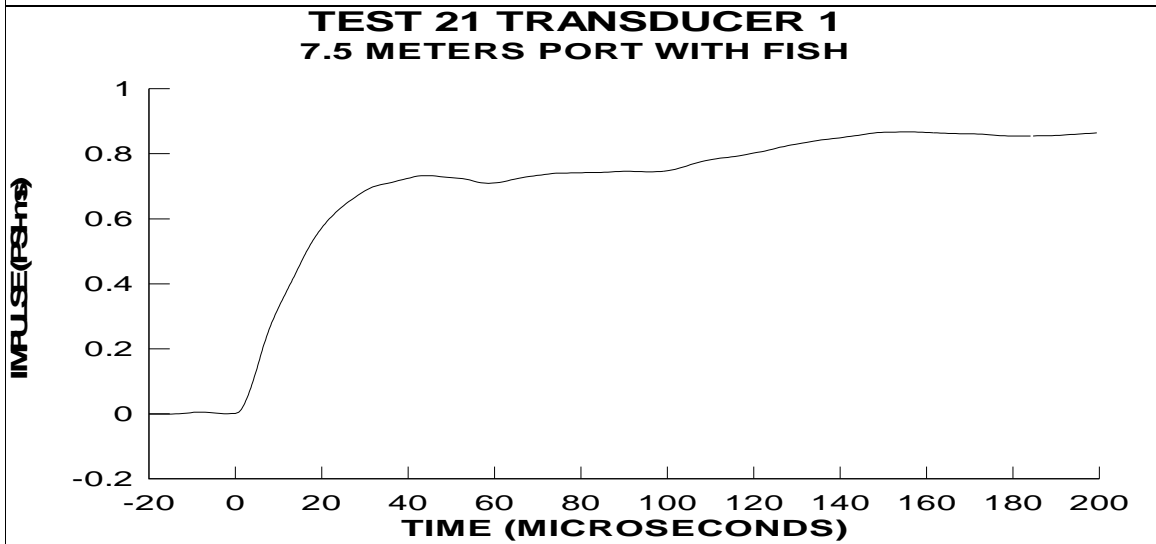
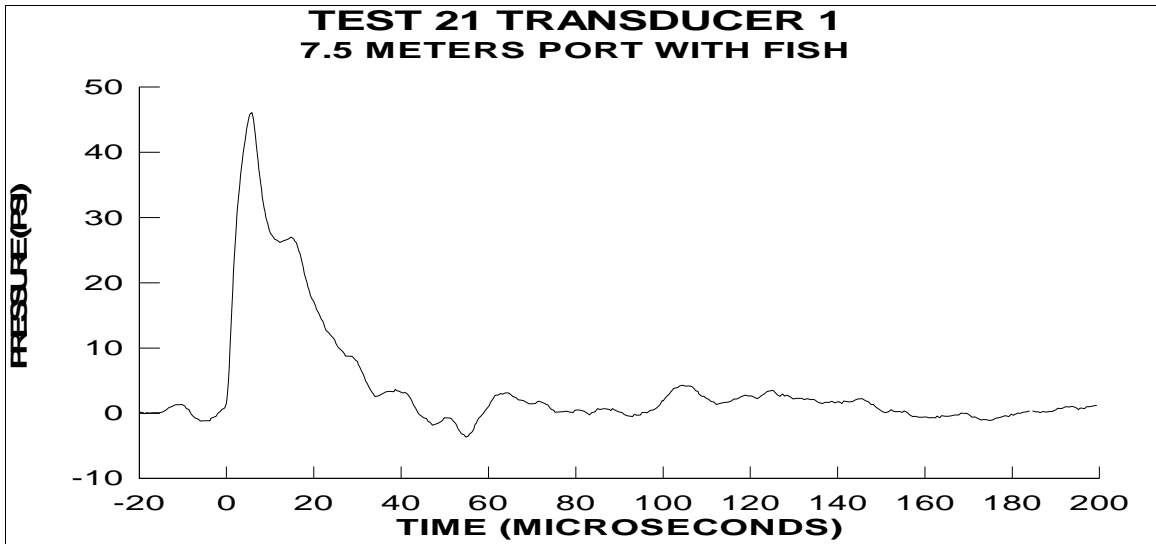


TEST 20 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH

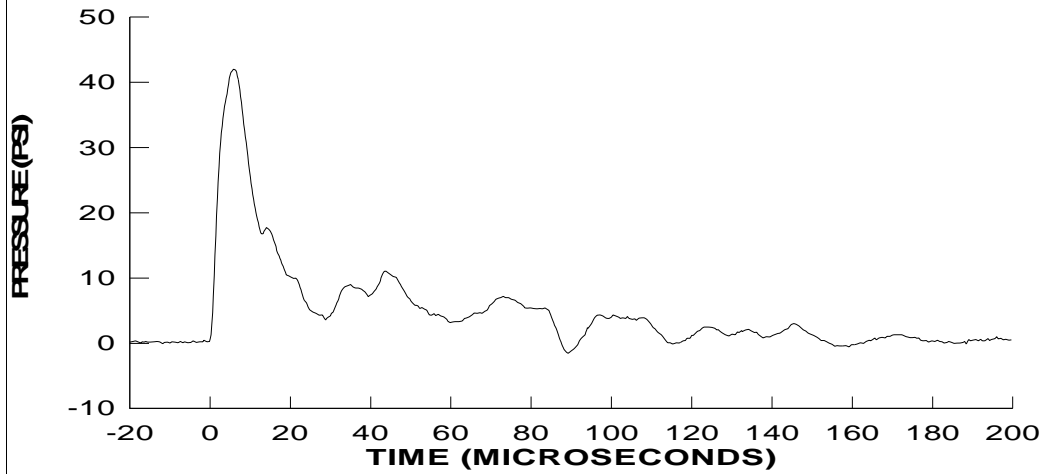


TEST 20 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH

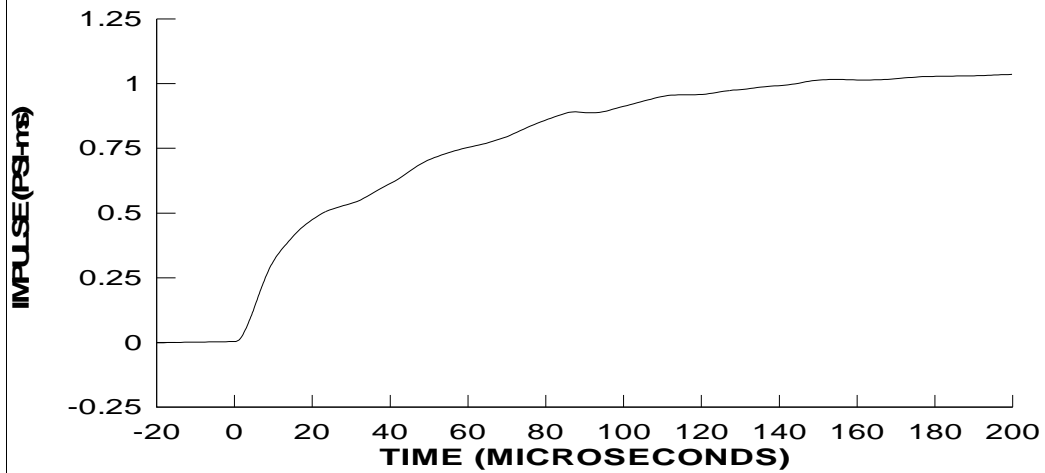




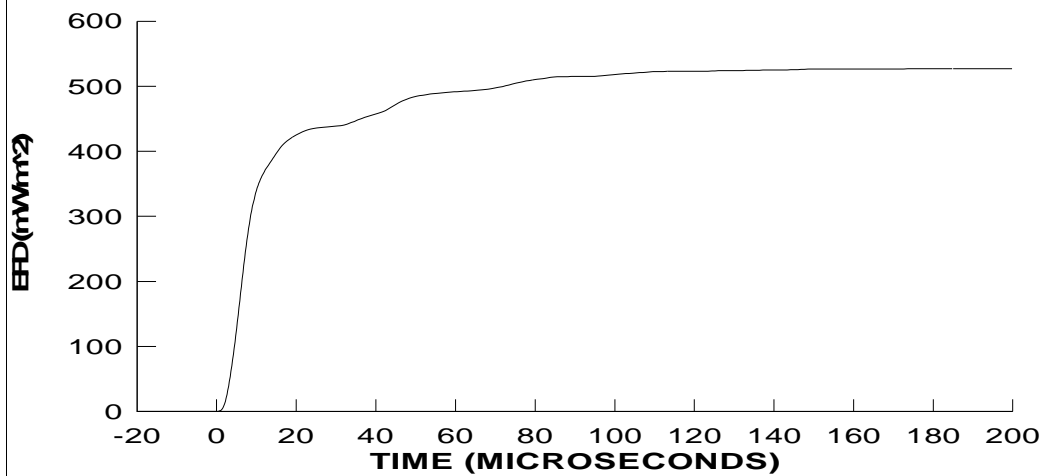
TEST 21 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



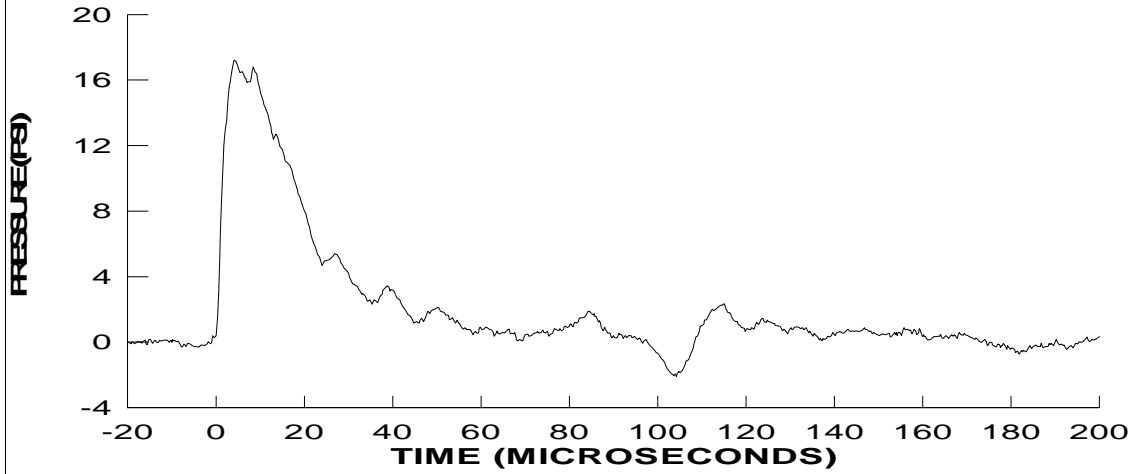
TEST 21 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



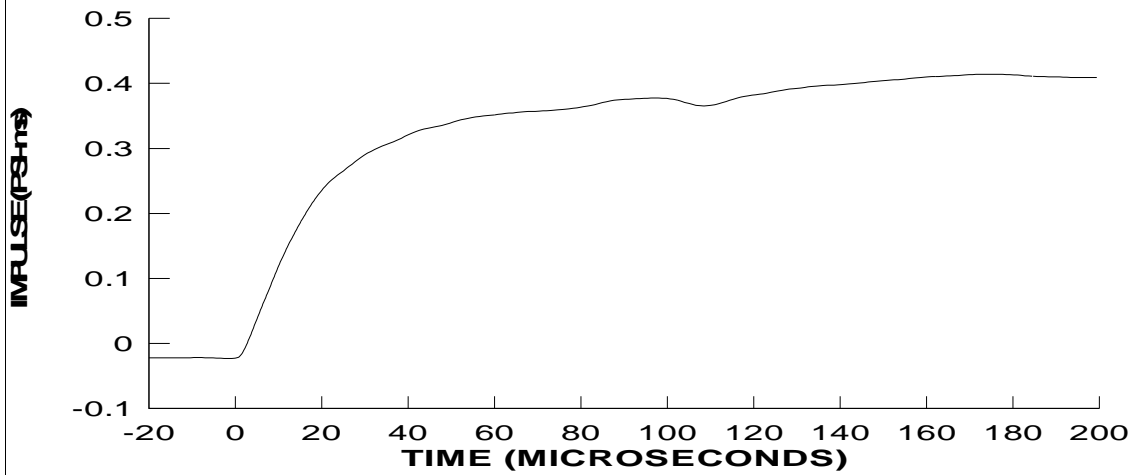
TEST 21 TRANSDUCER 2
7.5 METERS STARBOARD WITH FISH



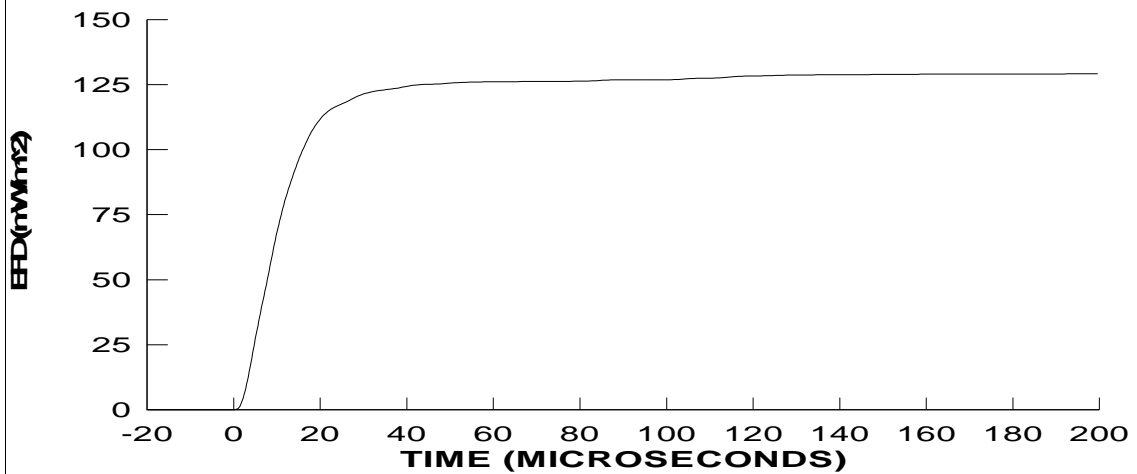
**TEST 22 TRANSDUCER 1
17 METERS PORT WITH FISH**



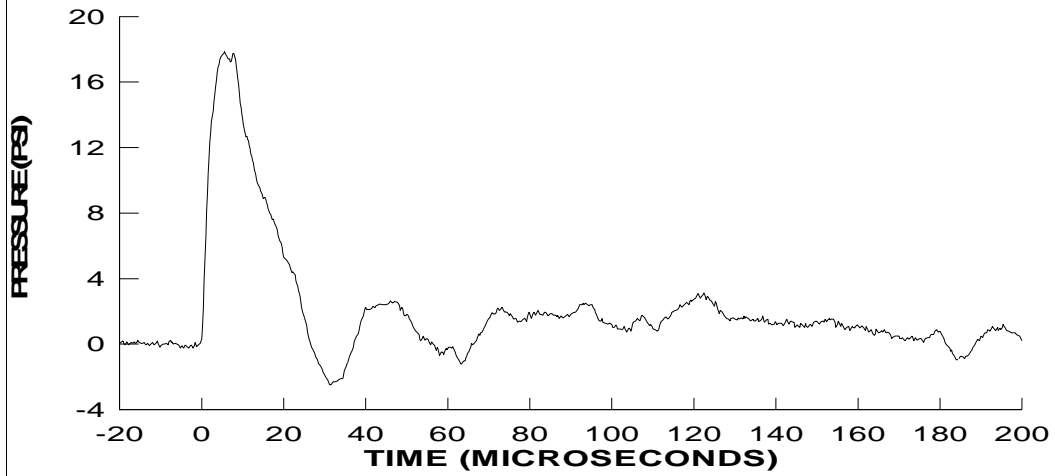
**TEST 22 TRANSDUCER 1
17 METERS PORT WITH FISH**



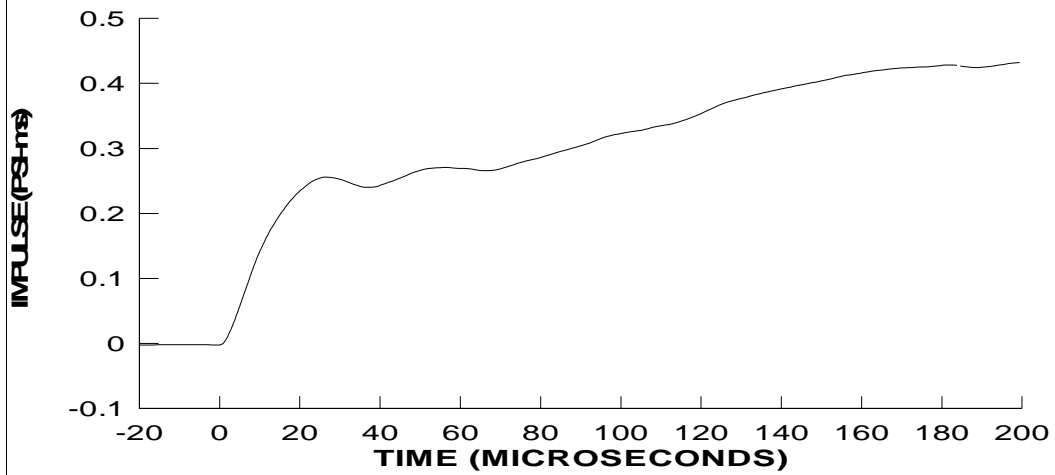
**TEST 22 TRANSDUCER 1
17 METERS PORT WITH FISH**



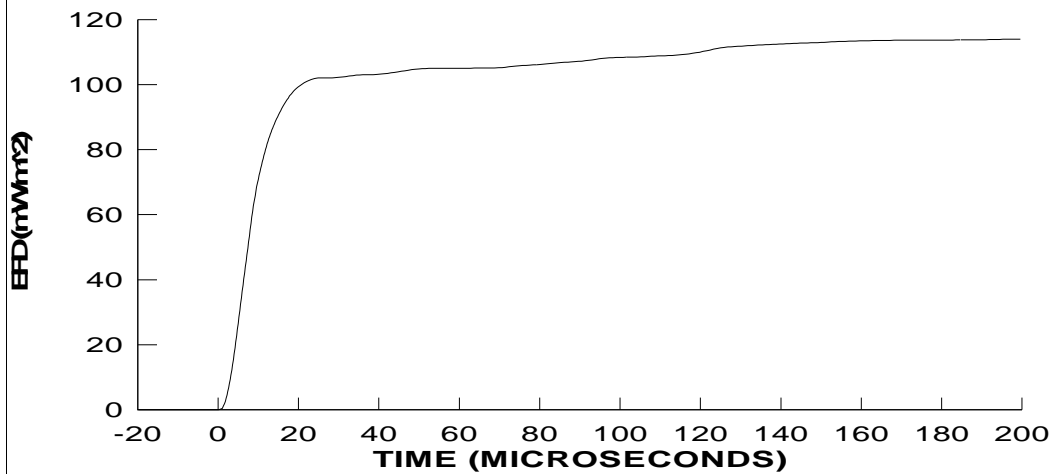
TEST 22 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



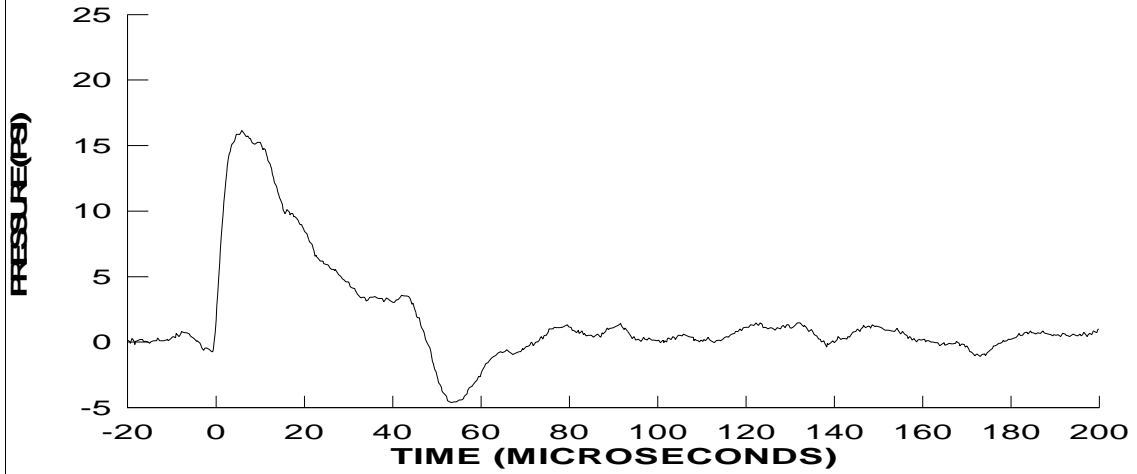
TEST 22 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



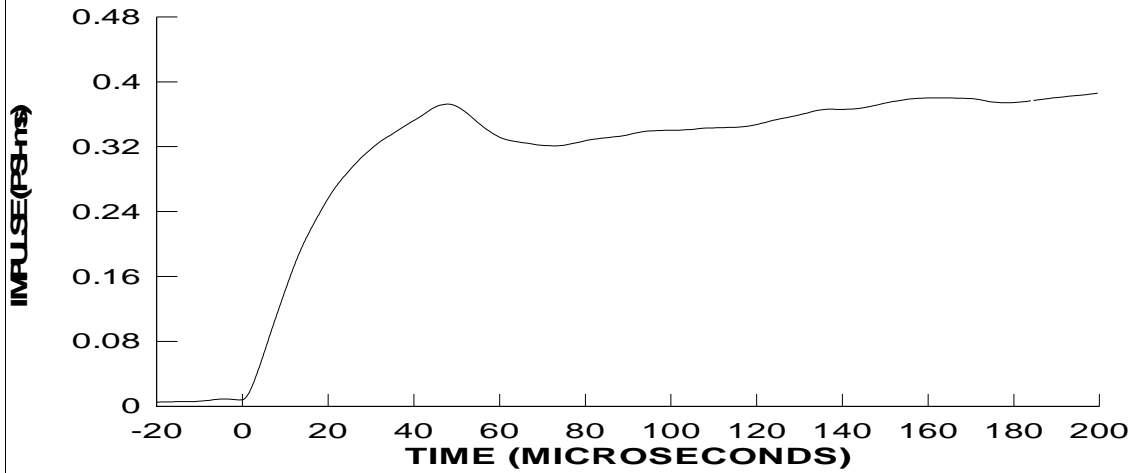
TEST 22 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



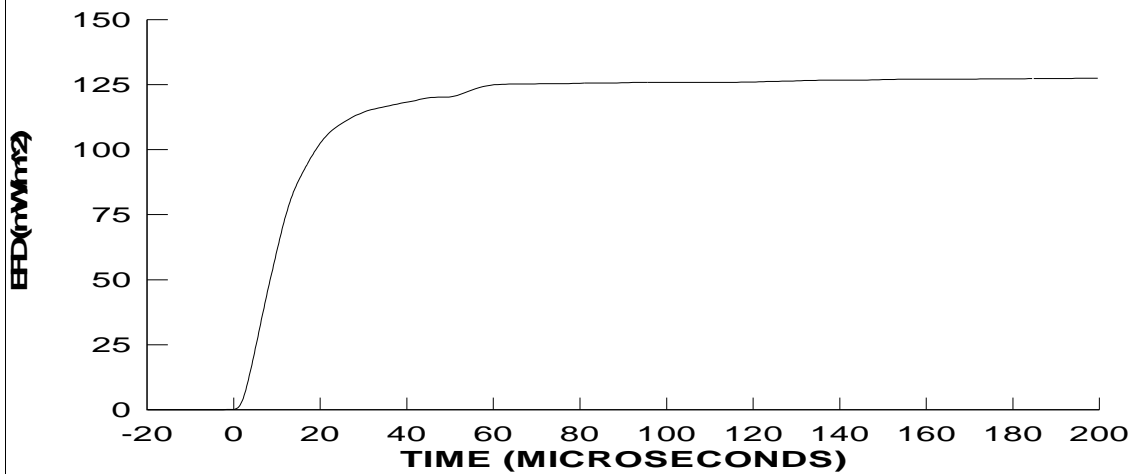
**TEST 23 TRANSDUCER 1
17 METERS PORT WITH FISH**



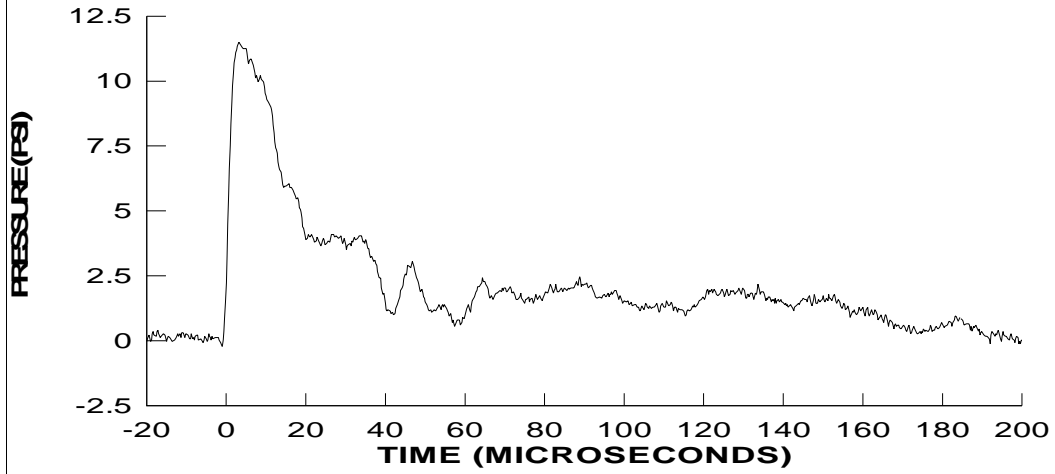
**TEST 23 TRANSDUCER 1
17 METERS PORT WITH FISH**



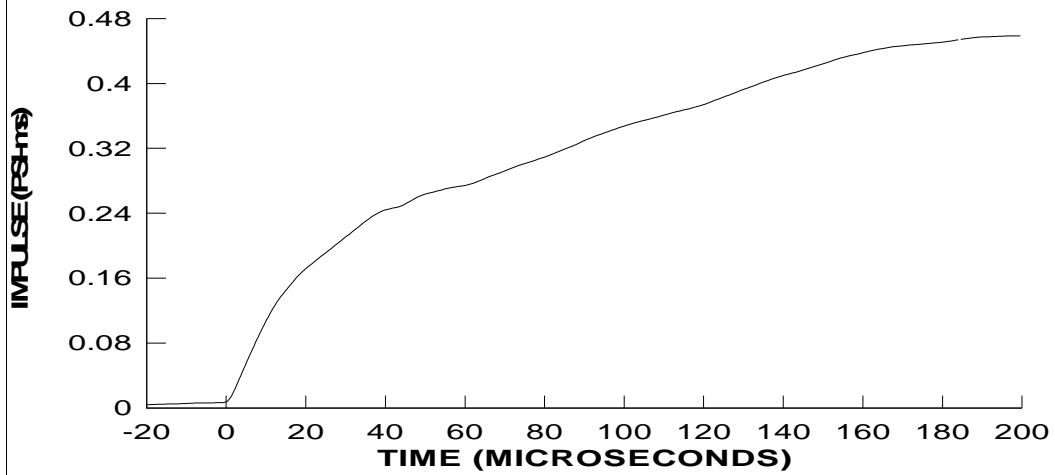
**TEST 23 TRANSDUCER 1
17 METERS PORT WITH FISH**



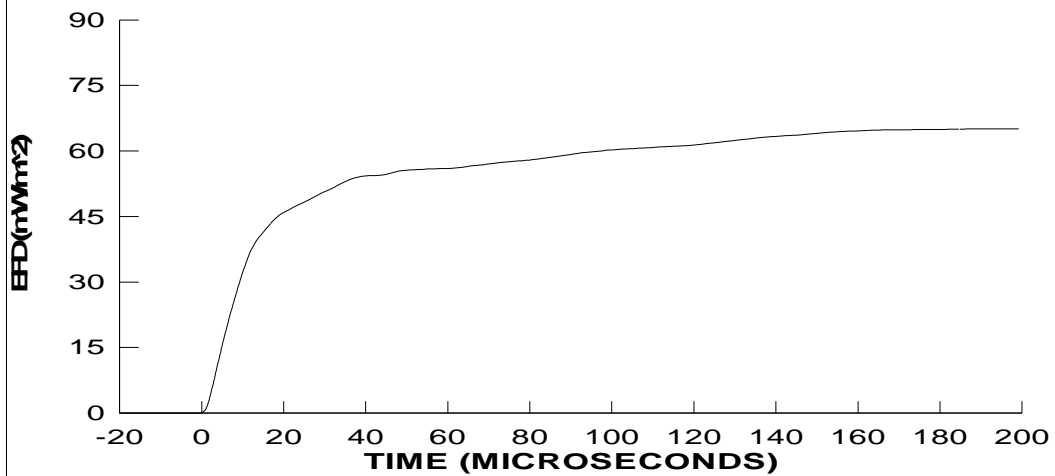
TEST 23 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



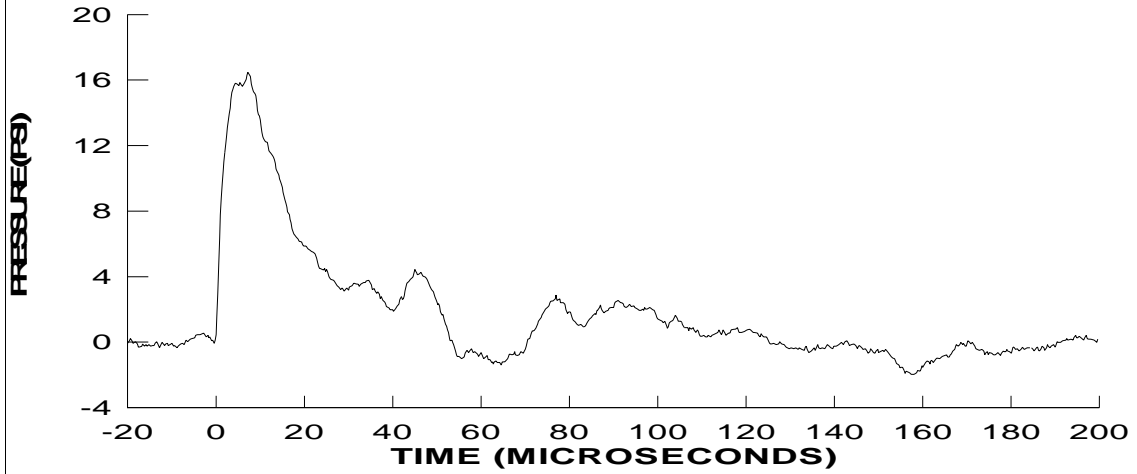
TEST 23 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



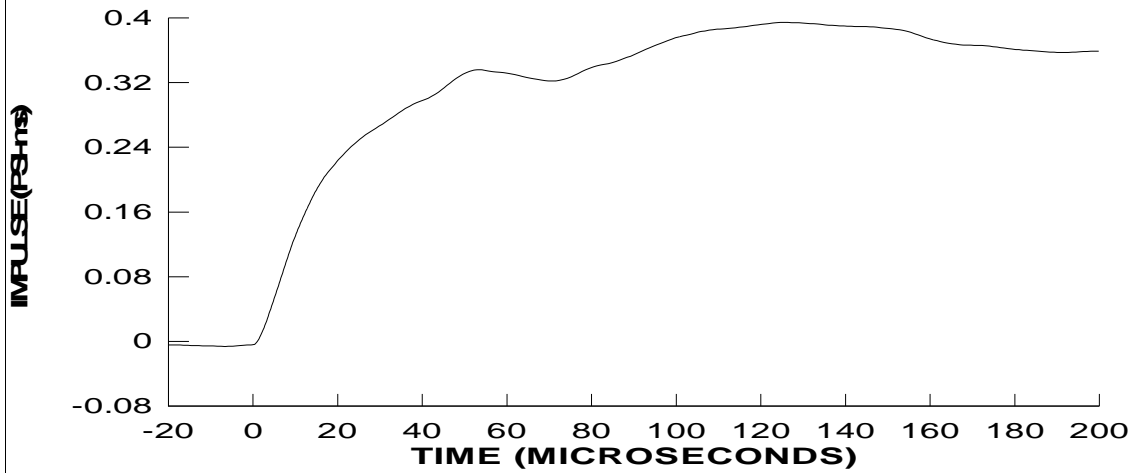
TEST 23 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



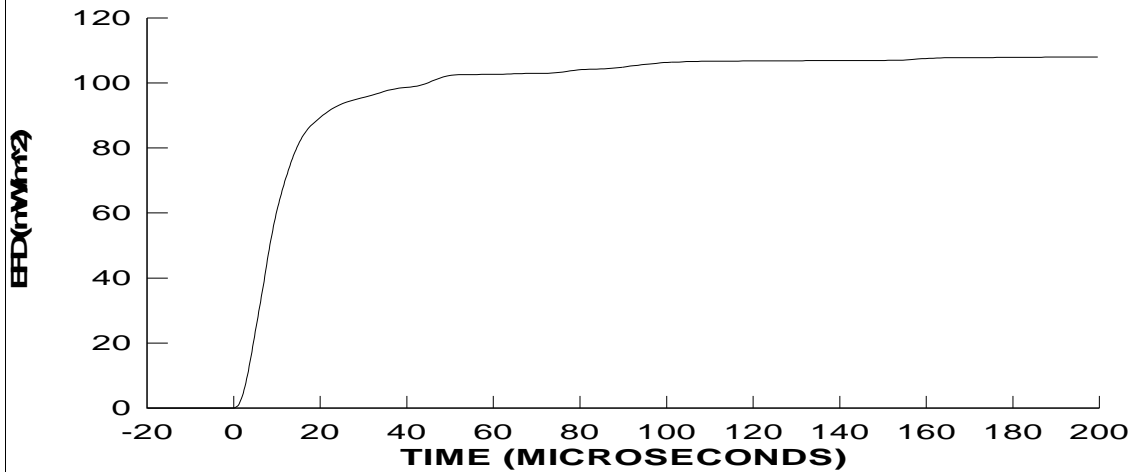
**TEST 24 TRANSDUCER 1
17 METERS PORT WITH FISH**



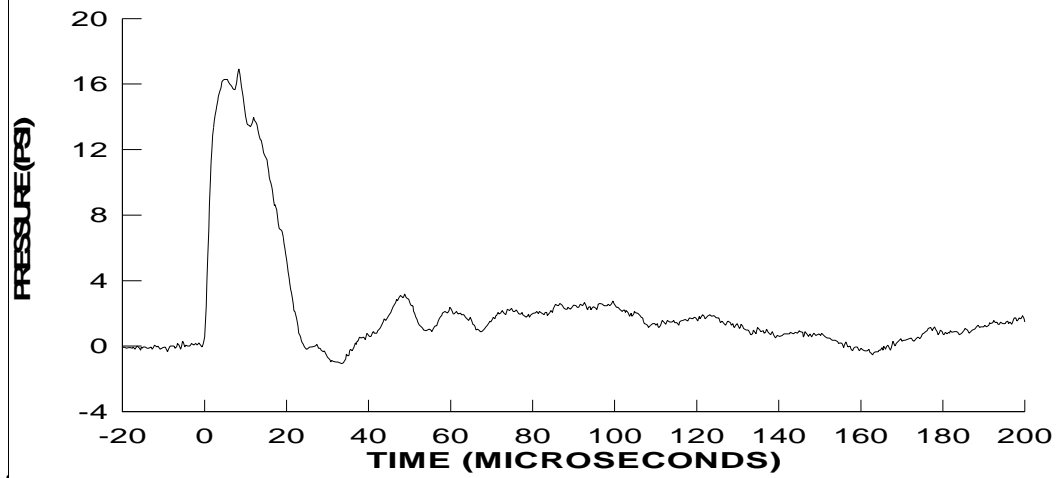
**TEST 24 TRANSDUCER 1
17 METERS PORT WITH FISH**



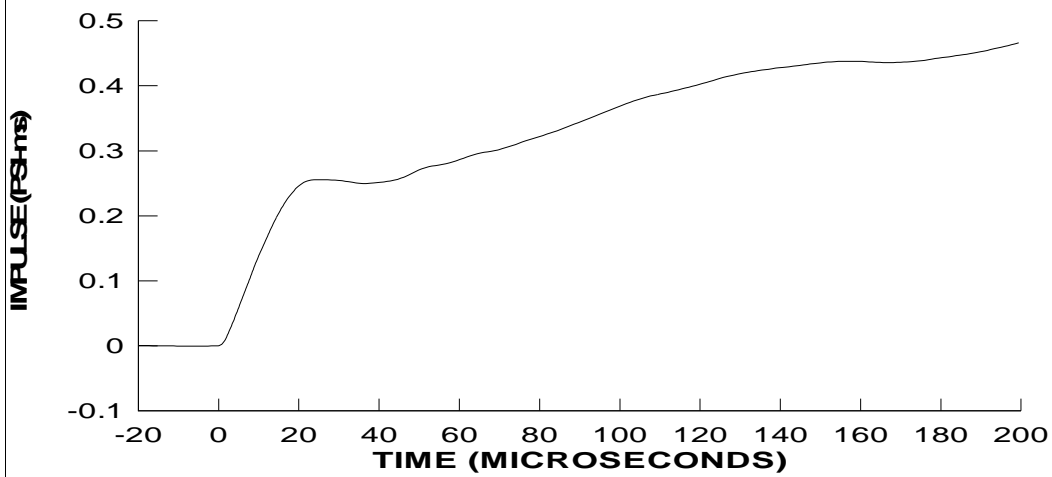
**TEST 24 TRANSDUCER 1
17 METERS PORT WITH FISH**



TEST 24 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



TEST 24 TRANSDUCER 2
17 METERS STARBOARD WITH FISH



TEST 24 TRANSDUCER 2
17 METERS STARBOARD WITH FISH

